AFP

ELISA Test for the Quantitative Determination of Alpha-Fetoprotein in Human Serum

Package Size

[REF]	52010	96 Tests	Complete Test Kit

[IVD]

Intended Use

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of 70 kDa synthesised during foetal development by the hepatocytes, yolk sac and to a lesser extent by the gastrointestinal tract. During pregnancy, AFP produced by the foetus is secreted into the amniotic fluid and the maternal circulatory system.

It has been established that gross defects of the foetus such as anencephaly and spina bifida can result in abnormally high AFP concentrations in maternal serum and amniotic fluid.[1] Abnormally low AFP concentrations in maternal serum are found in Down's syndrome pregnancies.[2,3] This test, however, has not been clinically evaluated for diagnosis of Down's syndrome (triple testing).

This AFP ELISA is intended for tumour diagnosis and especially in monitoring therapy as AFP together with CEA is among the most known carcinoembryonic antigens. Significant elevations of AFP levels can occur in sera of patients with tumours such as primary hepatoma and teratoma.[4,5,6] Primary hepatocellular carcinoma is based on liver cirrhosis, therefore monitoring of risk groups (HBsAg patients, alcoholic cirrhosis) is of high importance.

Principle - Direct Antigen EIA -

The HUMAN AFP ELISA is intended for professional use. The ELISA for direct antigen detection uses the high affinity of Biotin for Streptavidin, which has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and antibody-enzyme conjugate (monoclonal-AFP antibodies peroxidase-labelled and monoclonal anti-AFP antibodies biotinylated) are mixed to form the sandwich complex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidine. At the end of the incubation excess conjugate and unbound antigen are washed out. TMB/Substrate is added, a blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the AFP concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with streptavidin	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, yellowish AFP level: 0 (A), 5 (B), 25 (C), 50 (D), 250 (E), and 500 (F) ng/ml	
[CON]	13 ml	Antibody-Enzyme Conjugate (white cap) ready for use, coloured orange anti-AFP antibody (monoclonal, mouse) biotinylated and AFP antibody(monoclonal,mouse) HRP-labelled	
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for ca. 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	<0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

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Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.

- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or haemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	25	--
Specimens, Controls; in duplicate	--	25
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100

Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known AFP concentrations.

Validation of the Test

The test results are valid, if the following criteria are met:

The mean absorbance (OD) of [CAL] F ≥ 1.3 and [CAL] A ≤ 0.05.

The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

Detection of AFP is indicated in prenatal diagnostic for foetal defects and in diagnosis and therapeutic monitoring of primary hepatoma and teratoma.

AFP is the relevant tumour marker for primary hepatocellular carcinoma, the most frequent cancer world-wide with a low incidence in the industrialised countries. The sensitivity and specificity for primary hepatocellular carcinoma is 95-100%. The AFP concentration is in correlation to the differentiation of the tumour

Determination of the AFP value alone is not sufficient to assess cancer. It should be used in conjunction with other clinical manifestations and diagnostic parameters.

Expected Values

	AFP level
Normal healthy population (97-98%)	< 8.5 ng/ml
High risk patients suggesting hepatocellular carcinoma	100-350 ng/ml
Indication of hepatocellular carcinoma	> 350 ng/ml

The reference interval for AFP in serum of pregnant women is dependent on the gestational week and shows regional differences. Typical values for the median are as indicated.

Gestational Week	Median of AFP
16	33.4 ng/ml
17	37.5 ng/ml
18	44.8 ng/ml
19	50.8 ng/ml
20	58.1 ng/ml
21	68.2 ng/ml

Each laboratory should establish its own expected values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The HUMAN AFP ELISA test has an analytical sensitivity of about 0.025 ng. This is equivalent to the AFP concentration of 1 ng/ml specimen.

Specimens with AFP concentrations higher than 500 ng/ml should be diluted 1+9 with normal serum (AFP < 3 ng/ml) and re-assayed. Multiply the result by 10.

The assay is standardised in accordance with WHO 1st IS for human AFP (72/225): 1 ng/ml = 0.825 IU/ml.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-afp.pdf or

www.human-de.com/data/gb/vr/el-afp.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

1. Wald N., Cickle H., Lancet 29, 1323–1332 (1977)
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CMV IgG

ELISA Test for the Detection of IgG Antibodies to Cytomegalo Virus in Human Serum

Package Size

[REF]	51203	96 Tests	Complete Test Kit

[IVD]

Intended Use

The CMV IgG ELISA is intended for the detection of Immunoglobulin G (IgG) class antibodies to Cytomegalo Virus (CMV) in human serum.

CMV infections occur worldwide. About 50% of the general population is seropositive by the third decade of life. CMV is often a sexually transmitted disease, but may also be acquired by blood transfusions or via saliva, faeces, urine, or milk.

During pregnancy the fetus may become infected with CMV, and while the majority appear healthy at birth, up to 25% of these asymptomatically infected infants will show developmental disorders later (deafness, mental retardation). In normal adults CMV is usually asymptomatic, the most common form is mononucleosis. Typically, the patient presents with fever, chills, myalgia, and headache. The physician must rely on serological tests to distinguish CMV infection from similar symptoms caused by EBV or Toxoplasma gondii.

Principle - Classic EIA -

The HUMAN CMV IgG ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived CMV antigens (CMV-Ag). In the first incubation step corresponding specific anti-bodies (CMV-IgG-Ab) present in patient specimens or controls bind to the antigens at the solid phase. At the end of the incubation unbound components are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugated) is added which binds specifically to IgG class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the CMV-IgG-Ab concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) (Code CMV G) 8-well snap-off strips coated with CMV antigen (cell culture derived)	
[NC]	2.5 ml	CMV IgG Negative Control (green cap) human serum, pre-diluted, ready for use	
[PC]	2.5 ml	CMV IgG Positive Control (red cap) human serum (21 IU/ml of anti-CMV IgG activity), pre-diluted, ready for use (calibrated against the WHO 1st International Standard for detection of anti-CMV IgG, PEI code 136616/17)	
[DIL-G]	100 ml	Dilution Buffer IgG (white cap)	
5121		ready for use, coloured green	pH 6.5 ± 0.2
		phosphate buffer	10 mmol/l
		NaCl	8 g/l
		albumin	10 g/l
[CON]	12 ml	Anti-IgG Conjugate (white cap) ready for use, coloured red anti-human IgG (rabbit), peroxidase-conjugated	
[WS]20x]	50 ml	Washing Solution (white cap)	
5102		concentrate for about 1000 ml	pH 7.2 ± 0.2
		Tris buffer	10 mmol/l
		NaCl	8 g/l
[SUB]	13 ml	Substrate Reagent (black cap)	
5103		ready for use, colourless to bluish	pH 3.7 ± 0.2
		3,3', 5,5'-tetramethylbenzidin (TMB)	1.2 mmol/l
		hydrogen peroxide	3 mmol/l
[STOP]	15 ml	Stop Solution (red cap)	
5104		sulphuric acid, ready for use	0.5 mol/l
	2	Adhesive Strips	

Preservatives: total concentration < 0,1%

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/630–690 nm filters, deionised water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

[MIC] (Code: CMV G)

- sealed in an aluminium bag with a desiccant
- must be at room temperature before opening
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C
- Do not touch the upper rim or the bottom of the wells with fingers

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The general purpose reagents [DIL-G] 5121, [WS]20x] 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits. For IgG tests use only IgG dilution buffer [DIL-G] 5121.

All other reagents are specific for the individual package lot and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution [WASH]

- dilute [WS]20x] 5102 1 + 19 with fresh deionised water,
  e.g. 50 ml [WS]20x] 5102 + 950 ml = 1000 ml.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of Microtiter Strips.

P5: Run duplicates for controls. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P9: Always firmly close vials with the proper caps after use

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add [WASH] to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.				
Sample Preparation: Dilute the patient's sera 1 + 100 with [DIL-G] 5121, e.g. 10 µl serum + 1 ml [DIL-G] 5121, mix thoroughly. Diluted samples can be stored up to 48 h at 2...8°C before testing. Controls are ready for use.				
Step 1	*Well [µl]*			
	A1 Blank	B1/C1 [NC]	D1/E1 [PC]	F1... Sample
[NC] in duplicate	--	100	--	--
[PC] in duplicate	--	--	100	--
Diluted samples	--	--	--	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 2				
[CON]	--	100	100	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 3				
[SUB] 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
[STOP] 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.				
Measure the absorbance at 450 nm as soon as possible or within 30 min after terminating the reaction, using a reference wavelength of 630-690 nm (if available).				

The absorbance of controls and specimens is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN´s HumaReader or ELISYS lines). Patient samples are rated qualitatively by comparison with a cut-off value. Activity of anti-CMV IgG in patient samples can be quantified in international units per ml (IU/ml) based on the positive control.

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] in wells B1 and C1 (MNC) and [PC] in wells D1 and E1 (MPC) are calculated according to:

$$MNC = \frac{A_{450}\,(B1) + A_{450}\,(C1)}{2}, \quad MPC = \frac{A_{450}\,(D1) + A_{450}\,(E1)}{2}$$

$$\text{Cut-off value COV} = MNC + (0.1 \times MPC)$$

The test run may be considered valid provided that the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ 0.250
3. MPC ≥ 0.750
4. MPC : MNC ≥ 5

Interpretation of Results

A450 (patient) ≥ COV + 15%: anti-CMV-IgG-Ab-positive

A450 (patient) < COV - 15%: anti-CMV-IgG-Ab-negative

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

If an ELISA reader is not available a visual interpretation of results is possible:

- The substrate blank in well A1 should appear colourless.
- A specimen can be considered positive if the colour of the sample well is definitely stronger than the colour of the [NC] wells B1/C1.

Anti-CMV IgG activity in international units

Activity of anti-CMV IgG in positive specimens can be expressed in international units (IU/ml):

$$\text{Activity (anti-CMV IgG)} = \frac{A_{450}\,(\text{patient})}{MPC} \times 21.0\ \text{IU/ml}$$

Performance Characteristics

The assay has been standardised against the WHO 1st International Standard for detection of anti-CMV IgG, PEI code 136616/17.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-cmvg.pdf or

www.human-de.com/data/gb/vr/el-cmvg.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

Safety Notes

[STOP] Warning

~~- Hazard statements~~

H315 Causes skin irritation.

H319 Causes serious eye irritation.

~~[SUB] Danger~~

~~- Hazard statements~~

~~H360D May damage the unborn child.~~

~~- Precautionary statements~~

[NC] [PC] [DIL] [CON] [WASH][20x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.~~

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

Literature

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CMV IgM

ELISA Test for the Detection of IgM Antibodies to Cytomegalo Virus in Human Serum

Package Size

[REF]	51103	96 Tests	Complete Test Kit

[IVD]

Intended Use

CMV IgM is an IVD intended for the detection of IgM antibodies against cytomegalo virus in human serum. The test is qualitative and serves as an aid to diagnosis of cytomegalo infection or confirmation of immunological status of the patient exposed to cytomegalo virus antigen.

Clinical value

CMV infections occur worldwide. About 50% of the general population is seropositive by the third decade of life. CMV is often a sexually transmitted disease, but may also be aquired by blood transfusions or via saliva, feces, urine, or milk.

During pregnacy the fetus may be infected by CMV, and while the majority appear healthy at birth, up to 25% of these asymptomatically infected infants will show developmental disorders later (deafness, mental retardation). In normal adults infection with CMV is usually asymptomatic, but when symptoms do occur, the most common form is mononucleosis. Typically, the patient presents with fever, chills, myalgia, and headache. Often the physician must rely on serological tests to distinguish CMV infection from other infections and clinical syndromes which have similar symptoms.

Principle - Classic EIA -

The HUMAN CMV IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived CMV antigens (CMV Ag). In the first incubation step corresponding specific antibodies (CMV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to prevent rheumatoid factor (RF) interference and competition from specific IgG present in the specimen.

At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the CMV-IgM-Ab concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) (Code CMV M) 8-well snap-off strips coated with CMV antigen (cell culture derived)	
[NC]	2.5 ml	CMV IgM Negative Control (green cap) ready for use, human	
[PC]	2.5 ml	CMV IgM Positive Control (red cap) ready for use, human	
[DIL-M] 5111	100 ml	Dilution Buffer IgM (blue cap) ready for use, coloured green Phosphate buffer NaCl Albumin Anti-human-IgG (goat)	 pH 6.5 ± 0.2 10 mmol/l 8 g/l 10 g/l
[CON]	12 ml	Anti-IgM Conjugate (white cap) ready for use, coloured red Anti-human IgM (rabbit), peroxidase-conjugated	
[WS]20x] 5102	50 ml	Washing Solution (white cap) Concentrate for about 1000 ml Tris buffer NaCl	 pH 7.2 ± 0.2 10 mmol/l 8 g/l
[SUB] 5103	13 ml	Substrate Reagent (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide	 pH 3.7 ± 0.2 1.2 mmol/l ≤ 6 mmol/l
[STOP] 5104	15 ml	Stop Solution (red cap) Sulphuric acid, ready for use	 0.5 mol/l
	2	Adhesive Strips	

Preservatives: Total concentration < 0.1%

Additional materials required but not supplied with the kit

Micropipettes, ELISA washer (optional), microplate reader equipped with 450 nm or with 450/630–690 nm filters, deionised water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

[MIC] (Code: CMV M)

- sealed in an aluminium bag with a desiccant
- must be at room temperature before opening
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.
Reagents not in use should always be stored at 2...8°C.

Notes

The general purpose reagents [DIL-M] 5111, [WS]20x] 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits. For IgM tests use only IgM dilution buffer [DIL-M] 5111.

All other reagents are specific for the individual package lot and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution [WASH]

- dilute [WS]20x] 5102 1 + 19 with fresh deionised water, e.g. 50 ml [WS]20x] 5102 + 950 ml = 1000 ml.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of Microtiter Strips.

P5: Run duplicates for controls. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. If more than 1 plate is used, repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P9: [DIL-M] – turbidity after addition of the sample has no influence on the results.

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add [WASH] to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.				
Sample Preparation: Dilute the patient's sera 1 + 100 with [DIL-M] 5111, e.g. 10 µl serum + 1 ml [DIL-M] 5111, mix thoroughly (see P9). Incubate diluted samples at least 5 min. prior to further processing. Diluted samples can be stored up to 24 h at 2...8°C before testing. Controls are ready for use.				
Step 1	*Well [µl]*			
	A1 Blank	B1/C1 [NC]	D1/E1 [PC]	F1... Sample
[NC] in duplicate	--	100	--	--
[PC] in duplicate	--	--	100	--
Diluted samples	--	--	--	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 2				
[CON]	--	100	100	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 3				
[SUB] 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
[STOP] 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.				
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

Readers and Automated Analyzers

Validated settings for HUMAN ELISA microplate readers (HumaReader) or automated HUMAN ELISA analyzers (ELISYS line) are preinstalled or can be obtained from your local distributor. Application sheets for Human instruments with analyzer/assay specific handling and performance information are accessible via: www.human.de/aps-elisa.

For automated analysers, other than those provided by HUMAN, follow section Pipetting Scheme and ensure all requirements described in section Procedural Notes are followed. All protocols for automated analyzers must be fully validated prior usage.

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] in wells B1 and C1 (MNC) and [PC] in wells D1 and E1 (MPC) are calculated according to:

$$MNC = \frac{A_{450}\,(B1) + A_{450}\,(C1)}{2}; \quad MPC = \frac{A_{450}\,(D1) + A_{450}\,(E1)}{2}$$

$$\text{Cut-off value COV} = MNC + (0.2 \times MPC)$$

The test run may be considered valid provided that the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ 0.250
3. MPC ≥ 0.400
4. MPC : MNC ≥ 3

Interpretation of Results

A_{450} (patient) ≥ COV + 15%: anti-CMV-IgM-Ab-positive

A_{450} (patient) < COV - 15%: anti-CMV-IgM-Ab-negative

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-cmvm.pdf or

www.human-de.com/data/gb/vr/el-cmvm.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Limitations

A weak cross reactivity can occur with ANA and RF positive samples. In rare cases CMV IgG and Parvovirus B19 IgM positive samples may cause a weak cross reactivity. Strong cross reactivity was observed with HSV IgM positive serum. The analysis showed no cross reactivity in cases of: IM (EBV IgM positive), VZV IgM, Toxoplasma IgM, Rubella IgM and anti–HBc IgM positive samples.

Safety Notes

[STOP] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

~~[SUB] **Danger**~~

~~H360D May damage the unborn child.~~

[NC] [PC] [WS20x] [DIL-G] [CON] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/ regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. However all patient specimens and controls should be handled as potentially infectious.~~

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

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H. Pylori IgA ELISA

Indirect ELISA for the Detection of IgA Antibodies to *Helicobacter pylori* in Human Serum or Plasma

Package Size

[REF]	51322	96 Tests	Complete Test Kit
[IVD]			

Intended Purpose

HUMAN H. Pylori IgA ELISA is a colorimetric enzyme immunoassay for the qualitative and quantitative detection of IgA-class antibodies to the bacterium *Helicobacter pylori* (*H. pylori*) in human serum or plasma. It is used as an aid in the diagnosis of *H. pylori* infection. HUMAN H. Pylori IgA ELISA is designed for manual use or processing with automated ELISA analysers. For laboratory professional use only.

Clinical Significance

Helicobacter pylori is a spiral gram-negative bacterium which colonises the human gastric mucosa of more than half of the world population. An infection with *H. pylori* is associated with significant risk of duodenal ulcers and stomach cancer. More than 80% of peptic ulcers and about 75% of non-cardia gastric cancer (NCGC) cases are related to an infection with *H. pylori*. Accurate diagnosis of an infection with *H. pylori* is crucial for the management of related diseases. Immunoassays detecting IgA- and IgG-class antibodies to the bacterium *H. pylori* are the most useful non-invasive methods for providing a specific serological diagnosis.

Principle - Indirect Antibody EIA

HUMAN H. Pylori IgA ELISA, a colorimetric enzyme immunoassay for indirect detection of IgA antibodies to *H. pylori*, makes use of specific inactivated recombinant *Helicobacter pylori* (CagA) antigen coated on the surface of microtiter wells. IgG-class antibodies and rheumatoid factor (RF) are precipitated in the specimen by the IgG-RF-remover to prevent non-specific interference. In step 1, anti-*H. pylori* IgA present in diluted specimen bind to immobilized *H. pylori* Ag. In step 2, unbound specimen components are washed out and the wells are incubated with a conjugate of anti-human-IgA antibodies and the horseradish peroxidase (HRP) enzyme. In step 3, unbound conjugate is removed and TMB, a chromogenic substrate of the HRP enzyme, is added. The HRP moiety of bound conjugate converts colourless TMB to a water-soluble blue product. A pH drop stops the reaction and turns the product yellow. Colouration intensity is proportional to the concentration of anti-*H. pylori* IgA in the specimen. The optical density (OD) of controls, calibrators and specimens is determined by using microplate readers or automated ELISA analyzers. A specimen is classified reactive or non-reactive for anti-*H. pylori* IgA against a cut-off OD derived from calibrator 1 or after a quantitative interpretation based on a calibration curve.

Reagents and Contents

[MIC]	12	Microtiter strips (in strip holder) breakable 8-well strips coated with specific recombinant *Helicobacter pylori* (CagA) antigen	
[DIL]	100 ml	Dilution buffer (neutral cap) ready for use, dyed yellow phosphate buffer, BSA, tween 20 ProClin 300 %	pH 7.2 ± 0.2 0.03
[NC]	2.0 ml	H. Pylori IgA negative control (yellow cap) ready for use, dyed yellow Human serum without *H. pylori* antibodies phosphate buffer, BSA, Tween 20 ProClin 300 %	 0.03
[PC]	1.0 ml	H. Pylori IgA positive control (black cap) ready for use, dyed purple Human serum including *H. pylori* Antibodies phosphate buffer, BSA, Tween 20 ProClin 300 %	 0.03
[CAL-1]	2.0 ml	**H. Pylori IgA Calibrator-1** (green cap) ready for use, dyed green, Concentration: 15 U/mL ProClin 300 %	 0.03
[CAL-2]	1.0 ml	**H. Pylori IgA Calibrator-2** (blue cap) ready for use, dyed blue, Concentration: 50 U/mL ProClin 300 %	 0.03
[CAL-3]	1.0 ml	**H. Pylori IgA Calibrator-3** (red cap) ready for use, dyed red, Concentration: 150 U/mL ProClin 300 %	 0.03
[CON]	20 ml	Anti-IgA conjugate (white cap) ready for use, dyed red HRP-conjugated anti-human-IgA, BSA ProClin 300 %	 0.03
[WS]20x]	30 ml	Wash solution (20x) (black cap) concentrate for 600 ml TRIS buffer, tween 20 ProClin 300 %	 pH 6.5 ± 0.1 0.03
[SUB]	14 ml	Substrate reagent (yellow cap) ready for use, colourless, brown bottle. 3,3',5,5'-tetramethylbenzidine (TMB), hydrogen peroxide	
[STOP]	14 ml	Stop solution (red cap) sulphuric acid, ready for use	 0.2 mol/l
[IgG-REM]	6.5 ml	IgG-RF-remover (white cap) ready for use, dyed yellow phosphate buffer, BSA, tween 20, anti-human-IgG ProClin 300 %	 0.03
1		Adhesive foil	
1		Quick guide	
1		Plate layout	

Additional Materials Required but not Supplied with the Kit

Test tubes for sample dilution; micropipettes; fresh pipette tips; microplate incubator or shaker thermostatically set to 37 °± 1.0 °C; microplate reader equipped with 450 nm or with 450/620–690 nm filters; deionised water (diH_2O); ELISA washer (optional).

Readers and Automated Analysers

Process the assay manually (optionally integrating a microplate washer and/or dispenser in the procedure) and evaluate with an adequate microplate reader, or use a suitable automated ELISA analyzer. For automated analyzers, follow the Pipetting Scheme section and ensure that all requirements described in the Procedural Notes section have been satisfied. All protocols for automated analyzers must be fully validated by user laboratory.

Storage/Stability

Unopened at 2-8 °C	up to the stated expiration date
Opened at 2-8 °C	2 months
[WASH] at 2-8 °C	1 week

Reagent Preparation

Bring all reagents to room temperature (20...25 °C) before use. Reagents not in use should always be stored at 2...8 °C.

[MIC]

- Allow to reach 20…25 °C before opening!
- Return unused [MIC] to the re-sealable zip-lock bag and store it with desiccant at 2...8 °C.
- Do not touch the upper rim or the bottom of the wells with fingers.

Working wash solution [WASH]

- Dilute [WS]20x] 1+19 with fresh deionised water in a suitable container, e.g. 10 ml [WS]20x] + 190 ml diH_2O = 200 ml. If crystals are present in [WS]20x], heat to 37 °± 2.0 °C until completely dissolved. [WASH] has a pH value of 7.2 ± 0.2.

Specimens

- Un-pooled and undiluted human serum or plasma (anticoagulants: EDTA Li-heparin or citrate).
- Avoid haemolysis by separating as soon as possible whole blood specimens. Do not use highly lipemic, haemolysed or icteric specimens. Remove any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen before use. Do not use heated specimens.
- Specimens may be stored for 5 days at 2...8 °C or for longer (up to 12 months) at -20 °C. Freeze and thaw once only. Thawed specimen must be homogenised.
- Particulate matter and fibrin particles may yield reactive results. Avoid pipetting particulate matter.
- Incompletely coagulated sera and microbially-contaminated specimens should not be used.

Procedure

Follow the procedure exactly as described!

Procedural Notes

P1: Do not mix reagents from different lot numbers within a run unless they are general purpose reagents. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.
P2: Each sample, control and reagent must be pipetted with a single fresh tip.
P3: Do not use reagents that could be contaminated or look or smell different than usual.
P4: Record specimens and controls carefully on the spread sheet supplied with the kit.
P5: [MIC] - select the required number of microtiter strips.
P6: Do not let the wells dry once the assay has been started.
P7: Do not reuse the coated plates.
P8: Never use the same container for conjugate and solutions.
P9: Always pipette controls, samples and reagents in the same order and timing according to the assay procedure and without interruptions to minimize reaction time differences between wells. This is important for reproducible results.
P10: Avoid/remove air bubbles prior to incubation and reading of absorbance.
P11: Do not expose the reagents to excessive heat or sunlight during storage and test procedure.
P12: Do not use reagents without label or with damaged package.
P13: Always firmly close vials with the proper caps after use.
P14: Remove only reagents required for a run from stock solutions since they could come into contact with other contaminating solutions like patient specimens etc.
P15: Always store stock solutions at 2...8 °C when not in use; do not freeze the reagents.
P16: Do not run the test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals.
P17: Since the enzyme reaction is very sensitive to metal ions, do not allow any metal element to come into contact with conjugate or substrate solutions. Upon development of automated applications, the compatibility of any metal tips used for dispensing must be addressed in the validation.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision. The use of an automatic washer is strongly recommended.

W1: Remove the adhesive protective film from [MIC] if applied and transfer the contents of all wells into 5% sodium hypochlorite solution or another disinfectant.
W2: Add [WASH] to each well at the volume specified in the Pipetting scheme. Aspirate after a soak time of 30 s. Ensure that no liquid is left in the wells (use double aspiration in the final step where possible). Repeat this step as specified in the Pipetting Scheme.
W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Sample preparation:

Dilute patient's specimen 1+50 with [DIL]: 10 µl serum + 0.5 ml [DIL]. Mix thoroughly. Rock [IgG-REM] gently but thoroughly. Combine the diluted sample with [IgG-REM] 1+1: 60 µl diluted specimen + 60 µl [IgG-REM]. Mix thoroughly, let stand for at least 15 minutes and up to 2 hours at room temperature, mix thoroughly again.

[NC], [CAL1-3] and [PC] are ready for use, rock gently but thoroughly before use.

Step 1	*Well [µl]*					
	A1 [NC]	B1-C1 [CAL-1	D1 [CAL-2	E1 [CAL-3	F1 [PC]	G1… Sample
[NC]	100	--	--	--	--	--
[CAL-1]	--	100	--	--	--	--
[CAL-2]	--	--	100	--	--	--
[CAL-3]	--	--	--	100	--	--
[PC]	--	--	--	--	100	--
Diluted samples combined with [IgG-REM]	--	--	--	--	--	100
Mix carefully						
Seal [MIC] with adhesive foil						
Incubate for **60 min at 37 °C**						
Wash as described: W1, **5x** W2, W3						
[WASH]	300	300	300	300	300	300
Step 2						
[CON]	100	100	100	100	100	100
Incubate for **30 min at room temperature (20…25 °C)**						
Wash as described: W1, **5x** W2, W3						
[WASH]	300	300	300	300	300	300
Step 3						
[SUB]	100	100	100	100	100	100
Incubate for **15 min at 20…25°C** in the dark						
[STOP]	100	100	100	100	100	100
Mix carefully						
Measure the optical density at 450 nm within 30 min after terminating the reaction. Use a reference wavelength of 620 nm if available.						

Validation of assay run

The test run is valid if the following criteria are met:

1. **NC** < 0.2
2. 0.35 ≤ **CAL-1** (*Cut-off*) ≤ 0.85 (each value with [CAL-1])
3. 0.70 ≤ **CAL-2** ≤ 1.40
4. 1.40 ≤ **CAL-3** ≤ 2.80
5. 0.65 ≤ **PC** ≤ 3.00

Calculation for qualitative results:

Calculate Cut-off value (**COV**) according to:

$$COV = \frac{OD[B1] + OD[C1]}{2}$$

Interpretation of qualitative Results

A specimen is classified reactive, non-reactive or equivocal for anti-*H. Pylori* IgA by comparing the OD determined for the specimen (OD [spec]) to the COV. A ratio between OD [spec] and COV can be calculated to yield the S/Co (index) value.

OD	S/Co	Interpretation
OD [spec] < 0.9 x COV	below 0.9	Nonreactive
OD [spec] > 1.1 x COV	above 1.1	Reactive
OD [spec] ≥ 0.9 x COV and OD [spec] ≤ 1.1 x COV	0.9 – 1.1	Equivocal

Calculation for quantitative results:

The results found in patient's sera can be expressed in units per ml (U/ml):

For the quantitative evaluation, the absorbance of the NC and CAL 1-3 are graphically plotted against their corresponding concentrations (0, 15, 50, 150 U/mL, respectively). From the resulting calibration curve, the concentration of the analyte in patient samples can be determined in correlation to their OD values.

For calculation of analyte concentration select an appropriate and validated curve fitting option (recommendation: point to point).

Interpretation of quantitative Results

A specimen is classified reactive, non-reactive or equivocal for anti-*H. Pylori* IgA by comparing its concentration in U/mL with the following reference values:

Sample Concentration (U/mL)	Interpretation
Conc. < 13.5	Nonreactive
Conc. > 16.5	Reactive
13.5 ≤ **Conc.** ≤ 16.5	Equivocal

Performance Characteristics

Calibration/Traceability

No international standard or other certified reference material for the biological activity of human IgA antibodies to *H. Pylori* is available.

Precision

HUMAN H. Pylori IgA ELISA features a repeatability in the range of %CV: 2.4% to 9.2% (%CV determined with 12 negative and positive samples in 20 replicates each) and a reproducibility in the range of %CV: 1.4% to 12.5% (%CV determined with 3 samples assayed in duplicate in 10 independent runs). The lot-to-lot variability of the assay was assessed by testing 3 samples with 3 different lots of Human H. Pylori IgA ELISA resulting in %CV range: 2.5% to 8.8%.

Interference

Common endogenous substances were evaluated for potential interference with the assay up to the following concentrations: bilirubin 0.5 mg/ml, haemoglobin 4.0 mg/ml, triglycerides 30 mg/ml. No interference was detected.

Cross-reactivity

HUMAN H. Pylori IgA ELISA showed no cross-reactivity to Helicobacter pylori IgG, Bordetella IgA, Chlamydia trachomatis IgA, Toxoplasma gondii IgA, Ureaplasma IgA, Mycoplasma hominis IgA, VZV IgA, Brucella abortus, Candida albicans, Treponema pallidum, Mycoplasma pneumonia, Echinococcus and Fasciola.

Sensitivity and Specificity

Diagnostic performance of HUMAN H. Pylori IgA ELISA has been determined by comparison to a predicate CE-marked H. Pylori IgA ELISA on a pool of 86 samples.

			Reference Mikrogen H.pylori IgA ELISA	
		n=86	+ 41	- 45
HUMAN H. Pylori IgA ELISA	+	41	41	0
	–	45	0	45

Overall agreement:	100.0% (95% CI: 95.80% to 100.00%)
Sensitivity (agreement positive results):	100.0% (95% CI: 91.40% to 100.00%)
Specificity (agreement negative results):	100.0% (95% CI: 92.13% to 100.00%)

Limitations

– As with all diagnostic tests, the results should be interpreted with due consideration of other laboratory findings and of the clinical status of the patient. A positive result for anti-*H. pylori* IgA may have to be confirmed by a national reference laboratory in compliance with national guidelines. A negative result does not preclude the possibility of exposure to or infection with *H. Pylori* bacterium.

Safety Notes

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious.

[STOP] Warning

H315 Causes skin irritation.
H319 Causes serious eye irritation.
[NC] [CAL-1-3] [PC] [DIL] [CON] [WS]20x] [SUB] [STOP] [IgG-REM]
P234 Keep only in original packaging.
P260 Do not breathe dust/fume/gas/mist/vapours/spray.
P262 Do not get in eyes, on skin, or on clothing.
P281 Use personal protective equipment as required.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 If eye irritation persists: Get medical advice/attention.
P401 Store in accordance with local/regional/national/international regulations.
P501 Dispose of contents/container in accordance with local/regional/ national/ international regulations.

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H. Pylori IgG ELISA

Indirect ELISA for the Detection of IgG Antibodies to *Helicobacter pylori* in Human Serum or Plasma

Package Size

[REF]	51222	96 Tests	Complete Test Kit
[IVD]			

Intended Purpose

HUMAN H. Pylori IgG ELISA is a colorimetric enzyme immunoassay for the quantitative and qualitative detection of IgG-class antibodies to the bacterium *Helicobacter pylori* (*H. pylori*) in human serum or plasma. It is used as an aid in the diagnosis of *H. pylori* infection. HUMAN H. pylori IgG ELISA is designed for manual use or processing with automated ELISA analysers. For laboratory professional use only.

Clinical Significance

Helicobacter pylori is a spiral gram-negative bacterium which colonises the human gastric mucosa of more than half of the world population. An infection with *H. pylori* is associated with significant risk of duodenal ulcers and stomach cancer. More than 80% of peptic ulcers and about 75% of non-cardia gastric cancer (NCGC) cases are related to an infection with *H. pylori*. Accurate diagnosis of an infection with *H. pylori* is crucial for the management of related diseases. Immunoassays detecting IgA- and IgG- class antibodies to the bacterium *H. pylori* are the most useful non-invasive methods for providing a specific serological diagnosis.

Principle - Indirect Antibody EIA

HUMAN H. Pylori IgG ELISA, a colorimetric enzyme immunoassay for indirect detection of IgG antibodies to *H. pylori*, makes use of specific inactivated recombinant *Helicobacter pylori* (Cag A) antigen coated on the surface of microtiter wells. In step 1, anti-*H. pylori* IgG present in diluted specimen bind to immobilized *H. pylori* Ag. In step 2, unbound specimen components are washed out and the wells are incubated with a conjugate of anti-human-IgG antibodies and the horseradish peroxidase (HRP) enzyme. In step 3, unbound conjugate is removed and TMB, a chromogenic substrate of the HRP enzyme, is added. The HRP moiety of bound conjugate converts colourless TMB to a water-soluble blue product. A pH drop stops the reaction and turns the product yellow. Colouration intensity is proportional to the concentration of anti-*H. pylori* IgG in the specimen.

The optical density (OD) of controls, calibrators and specimens is determined by using microplate readers or automated ELISA analyzers. A specimen is classified reactive or non-reactive for anti-*H. pylori* IgG against a cut-off OD derived from calibrator 1 or after a quantitative interpretation based on a calibration curve.

Reagents and Contents

[MIC]	12	Microtiter strips (in strip holder) breakable 8-well strips coated with specific recombinant *Helicobacter pylori* (CagA) antigen	
[DIL]	100 ml	Dilution buffer (neutral cap) ready for use, dyed yellow phosphate buffer, BSA, tween 20 ProClin 300	pH 7.2 ± 0.2 0.03 %
[NC]	2.0 ml	H. Pylori IgG negative control (yellow cap) ready for use, dyed yellow Human serum without *H. pylori* antibodies phosphate buffer, BSA, Tween 20 ProClin 300	 0.03 %
[PC]	2.0 ml	H. Pylori IgG positive control (black cap) ready for use, dyed purple Human serum including *H. pylori* Antibodies phosphate buffer, BSA, Tween 20 ProClin 300	 0.03 %
[CAL-1]	2.0 ml	**H. Pylori IgG Calibrator-1** (green cap) ready for use, dyed green, Concentration: 15 U/mL ProClin 300	 0.03 %
[CAL-2]	2.0 ml	**H. Pylori IgG Calibrator-2** (blue cap) ready for use, dyed blue, Concentration: 75 U/mL ProClin 300	 0.03 %
[CAL-3]	2.0 ml	**H. Pylori IgG Calibrator-3** (red cap) ready for use, dyed red, Concentration: 150 U/mL ProClin 300	 0.03 %
[CON]	20 ml	Anti-IgG conjugate (white cap) ready for use, dyed red HRP-conjugated anti-human-IgG, BSA ProClin 300	 0.03 %
[WS]20x]	30 ml	Wash solution (20x) (black cap) concentrate for 600 ml TRIS buffer, tween 20 ProClin 300	 pH 6.5 ± 0.1 0.03 %
[SUB]	14 ml	Substrate reagent (yellow cap) ready for use, colourless, brown bottle. 3,3',5,5'-tetramethylbenzidine (TMB), hydrogen peroxide	
[STOP]	14 ml	Stop solution (red cap) sulphuric acid, ready for use	 0.2 mol/l
	1	Adhesive foil	
	1	Quick guide	
	1	Plate layout	

Additional Materials Required but not Supplied with the Kit

Test tubes for sample dilution; micropipettes; fresh pipette tips; microplate incubator or shaker thermostatically set to 37°± 1.0°C; microplate reader equipped with 450 nm or with 450/620–690 nm filters; deionised water (diH_2O); ELISA washer (optional).

Readers and Automated Analysers

Process the assay manually (optionally integrating a microplate washer and/or dispenser in the procedure) and evaluate with an adequate microplate reader, or use a suitable automated ELISA analyzer. For automated analyzers, follow the Pipetting Scheme section and ensure that all requirements described in the Procedural Notes section have been satisfied. All protocols for automated analyzers must be fully validated by user laboratory.

Storage/Stability

Unopened at 2-8 °C	up to the stated expiration date
Opened at 2-8 °C	2 months
[WASH] at 20-25 °C	1 week

Reagent Preparation

Bring all reagents to room temperature (20...25 °C) before use. Reagents not in use should always be stored at 2...8 °C.

[MIC]

- Allow to reach 20...25 °C before opening!
- Return unused [MIC] to the re-sealable zip-lock bag and store it with desiccant at 2...8 °C.
- Do not touch the upper rim or the bottom of the wells with fingers.

Working wash solution [WASH]

- Dilute [WS]20x] 1+19 with fresh deionised water in a suitable container, e.g. 10 ml [WS]20x] + 190 ml diH_2O = 200 ml. If crystals are present in [WS]20x], heat to 37°± 2.0°C until completely dissolved. [WASH] has a pH value of 7.2 ± 0.2.

Specimens

- Un-pooled and undiluted human serum or plasma (anticoagulants: EDTA, Li-heparin or citrate).
- Avoid haemolysis by separating as soon as possible whole blood specimens. Do not use highly lipemic, haemolysed or icteric specimens. Remove any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen before use. Do not use heated specimens.
- Specimens may be stored for 3 days at 2...8 °C or for longer (up to 17 months) at -20 °C. Freeze and thaw once only. Thawed specimen must be homogenised.
- Particulate matter and fibrin particles may yield reactive results. Avoid pipetting particulate matter.
- Incompletely coagulated sera and microbially-contaminated specimens should not be used.

Procedure

Follow the procedure exactly as described!

Procedural Notes

P1: Do not mix reagents from different lot numbers within a run unless they are general purpose reagents. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.
P2: Each sample, control and reagent must be pipetted with a single fresh tip.
P3: Do not use reagents that could be contaminated or look or smell different than usual.
P4: Record specimens and controls carefully on the spread sheet supplied with the kit.
P5: [MIC] - select the required number of microtiter strips.
P6: Do not let the wells dry once the assay has been started.
P7: Do not reuse the coated plates.
P8: Never use the same container for conjugate and solutions.
P9: Always pipette controls, samples and reagents in the same order and timing according to the assay procedure and without interruptions to minimize reaction time differences between wells. This is important for reproducible results.
P10: Avoid/remove air bubbles prior to incubation and reading of absorbance.
P11: Do not expose the reagents to excessive heat or sunlight during storage and test procedure.
P12: Do not use reagents without label or with damaged package.
P13: Always firmly close vials with the proper caps after use.
P14: Remove only reagents required for a run from stock solutions since they could come into contact with other contaminating solutions like patient specimens etc.
P15: Always store stock solutions at 2...8 °C when not in use; do not freeze the reagents.
P16: Do not run the test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals.
P17: Since the enzyme reaction is very sensitive to metal ions, do not allow any metal element to come into contact with conjugate or substrate solutions. Upon development of automated applications, the compatibility of any metal tips used for dispensing must be addressed in the validation.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision. The use of an automatic washer is strongly recommended.

W1: Remove the adhesive protective film from [MIC] if applied and transfer the contents of all wells into 5% sodium hypochlorite solution or another disinfectant.
W2: Add [WASH] to each well at the volume specified in the Pipetting scheme. Aspirate after a soak time of 30 s. Ensure that no liquid is left in the wells (use double aspiration in the final step where possible). Repeat this step as specified in the Pipetting Scheme.
W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Sample preparation:
Dilute patient's specimen 1+100 with [DIL]: 10 µl serum + 1.0 ml [DIL]. Mix thoroughly.
[NC], [CAL1-3] and [PC] are ready for use, rock gently but thoroughly before use.

Step 1	*Well [µl]*					
	A1 [NC]	B1 [CAL-1	C1 [CAL-2	D1 [CAL-3	E1 [PC]	F1… Sample
[NC]	100	--	--	--	--	--
[CAL-1	--	100	--	--	--	--
[CAL-2	--	--	100	--	--	--
[CAL-3	--	--	--	100	--	--
[PC]	--	--	--	--	100	--
Diluted samples	--	--	--	--	--	100
Mix carefully						
Seal [MIC] with adhesive foil						
Incubate for **60 min at 37 °C**						
Wash as described: W1, **5x** W2, W3						
[WASH]	300	300	300	300	300	300
Step 2						
[CON]	100	100	100	100	100	100
Incubate for **30 min at room temperature (20…25°C)**						
Wash as described: W1, **5x** W2, W3						
[WASH]	300	300	300	300	300	300
Step 3						
[SUB]	100	100	100	100	100	100
Incubate for **15 min at 20…25°C** in the dark						
[STOP]	100	100	100	100	100	100
Mix carefully						
Measure the optical density at 450 nm within 30 min after terminating the reaction. Use a reference wavelength of 620 nm if available.						

Validation of assay run

The test run is valid if the following criteria are met:

1. **NC** < 0.2
2. 0.35 ≤ **CAL-1** (*Cut-off*) ≤ 0.85
3. 0.90 ≤ **CAL-2** ≤ 2.10
4. 1.80 ≤ **CAL-3** ≤ 3.00
5. 0.65 ≤ **PC** ≤ 3.00

Calculation for qualitative results:

Cut-off value (**COV**) corresponds to the OD of [CAL-1]:

COV = OD B1

Interpretation of qualitative Results

A specimen is classified reactive, non-reactive or equivocal for anti-*H. pylori* IgG by comparing the OD determined for the specimen (OD [spec]) to the COV. A ratio between OD [spec] and COV can be calculated to yield the S/Co (index) value.

OD	S/Co	Interpretation
OD [spec] < COV	below 1.0	Nonreactive
OD [spec] > 1.2 x COV	above 1.2	Reactive
OD [spec] ≥ COV and OD [spec] ≤ 1.2 x COV	1.0 –1.2	Equivocal

Calculation for quantitative results:

The results found in patient's sera can be expressed in units per ml (U/ml):

For the quantitative evaluation, the absorbance of the NC and CAL 1-3 are graphically plotted against their corresponding concentrations (0, 15, 75, 150 U/mL, respectively). From the resulting calibration curve, the concentration of the analyte in patient samples can be determined in correlation to their OD values.

For calculation of analyte concentration select an appropriate and validated curve fitting option (recommendation: point to point).

Interpretation of quantitative Results

A specimen is classified reactive, non-reactive or equivocal for anti-*H. pylori* IgG by comparing its concentration in U/mL with the following reference values:

Sample Concentration (U/mL)	Interpretation
Conc. < 15.0	Nonreactive
Conc. > 18.0	Reactive
15.0 ≤ **Conc.** ≤ 18.0	Equivocal

Performance Characteristics

Calibration/Traceability

No international standard or other certified reference material for the biological activity of human IgG antibodies to *H. pylori* is available.

Precision

HUMAN H. Pylori IgG ELISA features a repeatability in the range of %CV: 2.5% to 9.7% (%CV determined with 12 negative and positive samples in 20 replicates each) and a reproducibility in the range of %CV: 0.3% to 8.2% (%CV determined with 3 samples, kit controls and calibrators assayed in duplicate in 10 independent runs using 2 different lots of HUMAN H. pylori IgG). The lot-to-lot variability of the assay was assessed by testing 3 samples with 3 different lots of Human H. Pylori IgG ELISA resulting in %CV range: 3.2% to 13.5%

Interference

Common endogenous substances were evaluated for potential interference with the assay up to the following concentrations: bilirubin 0.5 mg/ml, hemoglobin 4.0 mg/ml, triglycerides 20 mg/ml. No interference was detected.

Cross-reactivity

HUMAN H. Pylori IgG ELISA showed no cross-reactivity to Brucella abortus, Candida albicans, Chlamydia trachomatis, Mycoplasma pneumonia, Treponema pallidum, Echinococcus, Fasciola and Toxoplasma gondii.

Sensitivity and Specificity

Diagnostic performance of HUMAN H. Pylori IgG ELISA has been determined by comparison to a predicate CE-marked H. Pylori IgG ELISA on a pool of 88 samples.

			Reference Mikrogen H.pylori IgG ELISA	
			+	-
		n=88	60	28
HUMAN H. Pylori IgG ELISA	+	60	60	0
	–	28	0	28

Overall agreement:	100.0% (95% CI: 95.89% to 100.00%)
Sensitivity (agreement positive results):	100.0% (95% CI: 94.04% to 100.00%)
Specificity (agreement negative results):	100.0% (95% CI: 87.66% to 100.00%)

Limitations

– As with all diagnostic tests, the results should be interpreted with due consideration of other laboratory findings and of the clinical status of the patient. A positive result for anti-*H. pylori* IgG may have to be confirmed by a national reference laboratory in compliance with national guidelines. A negative result does not preclude the possibility of exposure to or infection with *H. pylori* bacterium.

Safety Notes

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious.

[STOP] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[NC] [CAL-1-3] [PC] [DIL] [CON] [WS]20x] [SUB] [STOP]

P234 Keep only in original packaging.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/ national/ international regulations.

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++++ Change of ¼ ++++ Please read marked text carefully! ++++

HSV IgM

ELISA Test for the Detection of IgM Antibodies to Herpes Simplex Virus in Human Serum

Package Size

[REF]	51126	96 Tests	Complete Test Kit

[IVD]

Intended Use

The HSV IgM ELISA is intended for the detection of Immunoglobulin M (IgM) class antibodies to Herpes Simplex virus in human serum.

HSV infections occur world-wide. The incubation period following exposure to the virus is about 1 week (range 2-26 days). Clinical symptoms include herpes genitalis and labialis.

HSV infection in new-borns (less then 6 weeks of age) is frequently devastating. Untreated, the mortality exceeds 65%. Many who survive exhibit developmental disabilities. Infection occurs most commonly during delivery as the neonate passes through the infected birth channel.

Principle - Classic EIA -

The HUMAN HSV IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived Herpes Simplex virus antigens (HSV-Ag). In the first incubation step corresponding specific antibodies (HSV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to prevent rheumatoid factor (RF) interference and competition from specific IgG present in the specimen.

At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the HSV-IgM-Ab concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) (Code HSV M) 8-well snap-off strips coated with HSV antigen (tpye 1 and type 2)	
[NC]	2.5 ml	HSV IgM Negative Control (green cap) ready for use, human	
[PC]	2.5 ml	HSV IgM Positive Control (red cap) ready for use, human	
[DIL-M] 5111	100 ml	Dilution Buffer IgM (blue cap) ready for use, coloured green Phosphate buffer NaCl Albumin Anti-human-IgG (goat)	 pH 6.5 ± 0.2 10 mmol/l 8 g/l 10 g/l
[CON]	12 ml	Anti-IgM Conjugate (white cap) ready for use, coloured red Anti-human IgM (rabbit), peroxidase-conjugated	
[WS]20x] 5102	50 ml	Washing Solution (white cap) Concentrate for about 1000 ml Tris buffer NaCl	 pH 7.2 ± 0.2 10 mmol/l 8 g/l
[SUB] 5103	13 ml	Substrate Reagent (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin (TMB) Hydrogen peroxide	 pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l
[STOP] 5104	15 ml	Stop Solution (red cap) Sulphuric acid, ready for use	 0.5 mol/l
	2	Adhesive Strips	

Preservatives: Total concentration < 0.1%

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/630-690 nm filters, deionised water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

[MIC] (Code: HSV M)

- sealed in an aluminium bag with a desiccant.
- must be at room temperature before opening,
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C.

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The general purpose reagents [DIL-M] 5111, [WS]20x] 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits. For IgM tests use only IgM dilution buffer [DIL-M] 5111.

All other reagents are specific for the individual package lot and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution **[WASH]**

- dilute [WS]20x] 5102 1+19 with fresh deionised water, e.g. 50 ml [WS]20x] 5102 + 950 ml = 1000 ml.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of Microtiter Strips.

P5: Run duplicates for controls. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P9: [DIL-M] – turbidity after addition of the sample has no influence on the results.

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add [WASH] to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.				
Sample Preparation: Dilute the patient's sera 1+100 with [DIL-M] 5111, e.g. 10 µl serum + 1 ml [DIL-M] 5111, mix thoroughly (see P9). Incubate diluted samples at least 5 min. prior to further processing. Diluted samples can be stored up to 24 h at 2...8°C before testing. Controls are ready for use.				
Step 1	*Well [µl]*			
	A1 Blank	B1/C1 [NC]	D1/E1 [PC]	F1... Sample
[NC] in duplicate	--	100	--	--
[PC] in duplicate	--	--	100	--
Diluted samples	--	--	--	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 2				
[CON]	--	100	100	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 3				
[SUB] 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
[STOP] 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.				
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN´s HumaReader or ELISYS line). Results for patient samples are obtained by comparison with a cut-off value.

Readers and automated analyzers

Validated settings for HUMAN ELISA microplate readers (HumaReader) or automated HUMAN ELISA analyzers (ELISYS line) are preinstalled or can be obtained from your local distributor. Application sheets for Human instruments with analyzer/assay specific handling and performance information are accessible via: www.human.de/aps-elisa.

For automated analyzers, other than those provided by HUMAN, follow section Pipetting Scheme and ensure all requirements described in section Procedural Notes are followed. All protocols for automated analyzers must be fully validated prior to usage.

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] in wells B1 and C1 (MNC) and [PC] in wells D1 and E1 (MPC) are calculated according to:

$$MNC = \frac{A_{450}(B1) + A_{450}(C1)}{2}; \quad MPC = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

Cut-off value COV = MNC + (0.2 x MPC)

The test run may be considered valid provided that the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ 0.350
3. MPC ≥ 0.700
4. MPC : MNC ≥ 3

Interpretation of Results

A_{450} (patient) ≥ COV + 15%: anti-HSV-IgM-Ab-positive

A_{450} (patient) < COV -15%: anti-HSV-IgM-Ab-negative

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Due to reactivation of latent infections with other viruses, related to the Herpes viridae family, positive HSV IgM results may occur in sera from patients with such infections. The possibility of infections with other members of the Herpes viridae family should therefore be investigated before interpretation of results.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-hsvm.pdf or

www.human-de.com/data/gb/vr/el-hsvm.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

Safety Notes

[STOP] Warning

~~- Hazard statements~~

H315 Causes skin irritation.

H319 Causes serious eye irritation.

~~[SUB] **Danger**~~

~~- Hazard statements~~

~~H360D May damage the unborn child.~~

~~- Precautionary statements~~

[NC] [PC] [DIL-M] [CON] [WS]20x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with

water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.~~

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

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++++ ¼ - Read carefully! ++++ New Assay design++++

HSV 1 IgG

ELISA Test for the Determination of IgG Antibodies to *Herpes Simplex Virus* type 1 in Human Serum and Plasma

Package Size

[REF]	51216	96 Tests	Complete Test Kit
[IVD]			

Intended purpose

HUMAN HSV 1 IgG ELISA is a colorimetric enzyme immunoassay for the qualitative (cut-off) detection of IgG class antibodies against *Herpes Simplex Virus type 1* (HSV 1) in human serum and plasma (heparin, EDTA, citrate). It is used as an aid in the diagnosis of HSV 1 infection. The test can further be performed on pregnant women as part of a prenatal screening. HUMAN HSV1 IgG is designed for manual processing as well as for automated use with ELISA analysers. For laboratory professional use only.

Principle- Classic EIA

The HUMAN HSV 1 IgG ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with recombinant gG1 protein. In the first incubation step corresponding specific antibodies (HSV 1-IgG-Ab) present in patient specimens or controls bind to the immobilized antigen at the solid phase. At the end of the incubation unbound components are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugated) is added which binds specifically to IgG class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the HSV 1-IgG-Ab concentration in the specimen.

The optical density (OD) of controls and specimens is determined by using microplate readers or automated ELISA analysers. A specimen is classified positive or negative against a controls-derived cut-off OD. The cut-off has been set in reference to in-house and external commercially available quality control samples.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) (Code HS1 G) breakable 8-well snap-off strips coated with recombinant gG1 protein	
[NC]	2.5 ml	Negative Control (green cap) ready for use Human serum Sodium azide Thimerosal	
[PC]	2.5 ml	Positive Control (red cap) ready for use Human serum Sodium azide Thimerosal	
[DIL-G] 5121	100 ml	Dilution Buffer IgG (white cap) ready for use, coloured green Phosphate buffer Sodium azide Thimerosal	pH 6.5 ± 0.2
[CON]	12 ml	Anti-IgG Conjugate (white cap) ready for use, coloured red Anti-human IgG (rabbit), peroxidase-conjugated	
[WS]20x] 5102	50 ml	Washing Solution (white cap) Concentrate for 1000 ml Tris buffer Thimerosal	pH 7.2 ± 0.2
[SUB] 5103	13 ml	Substrate Reagent (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin (TMB) Hydrogen peroxide	pH 3.7 ± 0.2
[STOP] 5104	15 ml	Stop Solution (red cap) Sulphuric acid, ready for use	0.5 mol/l
	2	Adhesive Strips	

Preservatives: total concentration < 0.1%

General-purpose reagents [DIL-G] 5121, [WS]20x] 5102, [SUB] 5103 and [STOP] 5104 are interchangeable between HUMAN ELISA lots and kits that share the same reagent designation. For IgG tests use only IgG dilution buffer [DIL-G] 5121.

All other reagents are specific for the individual package lot and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Additional materials recommended but not supplied with the kit

Test tubes for sample dilution, micropipettes, fresh pipette tips, ELISA washer (optional), microplate reader equipped with 450 nm or with 450/620–680 nm filters, deionised water (diH_2O).

Readers and automated analysers

Validated settings for the following HUMAN ELISA devices are preinstalled or can be obtained from your local distributor. Application sheets for HUMAN instruments with analyser/assay-specific handling and performance information are accessible via: www.human.de/aps-elisa

HUMAN ELISA instrument	Instrument type	REF
HumaReader Single plus	microplate reader	18000
HumaReader HS	microplate reader	16670
Elisys Uno	automated analyser	17350
Elisys Duo	automated analyser	17200

For automated analysers other than those provided by HUMAN follow the Pipetting scheme section and ensure all requirements described in the Procedural notes section have been satisfied. All protocols for automated analysers must be fully validated prior to usage.

Storage/stability

Unopened at 2…8 °C	up to the stated expiration date
Opened at 2…8 °C	60 days
[WASH] at 17…25 °C	60 days

Reagent preparation

Bring all reagents to 17...25°C before use. Reagents not in use should always be stored at 2...8 °C.

[MIC] (Code: HS1 G)

- Allow to reach 17...25°C before opening!
- Lost vacuum in the bag of the coated plate will not affect the performance of the test.
- Return unused [MIC] to the re-sealable zip-lock bag and store it with desiccant at 2...8 °C.
- Do not touch the upper rim or the bottom of the wells with fingers.

Working wash solution [WASH]

- Dilute [WS]20x] 1+19 with fresh deionised water in a suitable container, e.g. 10 ml [WS]20x] + 190 ml diH_2O = 200 ml.
- [WASH] should always be stored at 17…25°C.

Specimen

- Un-pooled and undiluted human serum and plasma with anticoagulants citrate, heparin or EDTA. Do not use specimens preserved with sodium azide.
- Do not use highly lipemic or hemolysed specimens.
- Specimens may be stored for 7 days at 2...8°C or longer at -20°C. Freeze and thaw once only**.** Thawed specimen must be homogenised.
- Particulate matter and fibrin particles may yield reactive results. Eliminate particulate matter by centrifugation or filtration.
- Incompletely coagulated sera and microbially-contaminated specimens should not be used.

Procedure

Follow the procedure exactly as described.

Procedural notes

P1: Do not mix reagents from different lot numbers within a run unless they are general purpose reagents. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of microtiter strips.

P5: Do not let the wells dry once the assay has been started.

P6: Do not reuse the coated plates.

P7: Never use the same container for conjugate and solutions.

P8: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the controls for each plate.

P9: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P10: Avoid/remove air bubbles prior to incubation and reading of absorbance.

P11: Do not expose the reagents to excessive heat or sunlight during storage and test procedure.

P12: Do not use reagents without label or with damaged package.

P13: Always firmly close vials with the proper caps after use.

P14: Remove only reagents required for a run from stock solutions since they could come into contact with other contaminating solutions like patient specimens etc.

P15: Always store stock solutions at 2...8 °C when not in use; do not freeze the reagents.

P16: Do not run the test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision. The use of an automatic washer is strongly recommended.

W1: Remove the adhesive protective film from [MIC] if applied and transfer the contents of all wells into 5% sodium hypochlorite solution or another disinfectant.

W2: Add [WASH] to each well at the volume specified in the Pipetting scheme. Aspirate after a soak time of 30 s. Ensure that no liquid is left in the wells (use double aspiration in the final step where possible). Repeat this step as specified in the Pipetting scheme.

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper. Residual volume lower than 10 µl is not critical for the assay.

Pipetting Scheme

Sample Preparation:

Dilute patient's sera 1+100 with [DIL-G] 5121: 10 µl serum + 1 ml [DIL-G] 5121 Mix thoroughly (15 s).

Diluted samples can be stored up to 48 h at 2...8°C before testing.

[NC], [PC] are ready for use, rock gently but thoroughly before use.

Step 1	*Well [µl]*			
	A1 Blank	B1/C1 [NC]	D1/E1 [PC]	F1... Sample
[NC] in duplicate	--	100	--	--
[PC] in duplicate	--	--	100	--
Diluted samples	--	--	--	100
Mix carefully				
Seal [MIC] with adhesive strips				
Incubate for 30 min at 17...25°C				
Wash as described: W1, **4x** W2, W3				
[WASH]	350	350	350	350
Step 2				
[CON]	--	100	100	100
Seal [MIC] with adhesive strips				
Incubate 30 min at 17...25°C				
Wash as described: W1, **5x** W2, W3				
[WASH]	350	350	350	350
Step 3				
[SUB] 5103	100	100	100	100
Incubate 15 min at 17...25°C in the dark (see P9)				
[STOP] 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.				
Measure the absorbance at 450 nm as soon as possible or within 30 min after terminating of the reaction. Use a reference wavelength of 620-680 nm if available.				

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] in wells B1 and C1 (MNC), [PC] in wells D1 and E1 (MPC) are calculated according to:

$$MNC = \frac{A_{450}\,(B1) + A_{450}\,(C1)}{2}; \quad MPC = \frac{A_{450}\,(D1) + A_{450}\,(E1)}{2}$$

Cut-off value COV = MNC + 0.1 x MPC

The test run is valid if the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ 0.250
3. MPC ≥ 0.750
4. MPC : MNC ≥ 5

If invalid, re-run the test paying careful attention to the procedure as described above.

Interpretation of Results

Result	Interpretation
A_{450} (patient) ≥ COV + 15%	anti-HSV 1-IgG-Ab-positive
A_{450} (patient) < COV -15%	anti-HSV 1-IgG-Ab-negative
A_{450} (patient) ≥ COV -15% and A_{450} (patient) < COV + 15%	equivocal: retest (patient monitoring is recommended in case of a reoccurring equivocal result to exclude unspecific reactions or cross reactions)

Due to physiological and analytical variations a patient result lying 15% above or below the calculated cut-off value is classified equivocal. It is recommended to re-assay such a sample in parallel with a fresh sample taken 7 to 14 days later. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the patient history and additional investigations. Repeatedly equivocal samples may be subjected to a confirmatory test.

For accurate typing it is recommended to isolate the virus and identify the antigen with type-specific antisera. Due to the reactivation of latent infections with other viruses related to the Herpes viridae family, positive HSV 1 IgG results may occur in sera from patients with such infections. The possibility of infections with other members of the Herpes viridae family should therefore be investigated before interpretation of results.

Performance Characteristics

Calibration/Traceability

No international standard or other certified reference material for the biological activity of human IgG antibodies to *Herpes Simplex Virus* type 1 is available.

Precision

Precision of HUMAN HSV 1 IgG was evaluated by using 3 serum samples with grey zone (+/-), low positive (LP) and high positive (HP) status. Assay precision was determined by testing each sample in duplicate, 2 runs per day, for 20 days, using 1 lot.

	Mean [S/CO]	Repeatability		Within-lab precision	
	(N=80)	SD	CV	SD	CV
Sample 1 (+/-)	1.5	0.09	6.2%	0.15	9.9%
Sample 2 (LP)	2.8	0.11	3.8%	0.24	8.6%
Sample 3 (HP)	4.0	0.11	2.7%	0.36	9.1%

Interference

Interfering substances were added to a known sample. No interference was detected for the respective substances up to following concentrations: bilirubin 40 mg/dl, haemoglobin 1000 mg/dl, triglycerides 2500 mg/dl, lipid (Lipovenös) 1000 mg/dl.

***Cross**-reactivity*

The assay has been tested and does not show cross-reactivity with HSV 2 IgG, other members of the herpes virus family (EBV IgG, CMV IgG, VZV IgG) and rheumatoid factor. Anti-nuclear antibodies (ANA) positive specimens can in some cases react positively in the HUMAN HSV 1 IgG ELISA.

Sensitivity and specificity

Diagnostic performance of HUMAN HSV 1 IgG has been determined with 121 samples using discrepant analysis with Novagnost HSV 1 IgG ELISA as reference assay.

			Reference (resolved)	
			+	-
		121	50	71
HUMAN HSV 1 IgG	+	52	50	2
	-	69	0	69

Overall agreement:	98.3%
Sensitivity (agreement positive results):	100.0%
Specificity (agreement negative results):	97.2%

Limitations

1. All protocols for automated analysers must be fully validated prior to usage.
2. Since the enzyme reaction is very sensitive to metal ions, do not allow any metal element to come into contact with conjugate or substrate solutions. Upon development of automated applications, the compatibility of any metal tips used for dispensing has to be addressed in the validation.
3. As with all diagnostic tests, the results should be interpreted with due consideration of other laboratory findings and of the clinical status of the patient. A negative result does not preclude the possibility of exposure to or infection with HSV type 1.

Safety notes

~~All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious.~~

~~[SUB] Danger~~

~~H360D May damage the unborn child.~~

[STOP] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[NC] [PC] [DIL-G] [CON] [WS]20x] [SUB] [STOP]

P234 Keep only in original packaging.

P260 Do not breath dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/ national/ international regulations.

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

References

1. Engvall, E., Perlmann, P., Immunochemistry **8**, 871-874 (1971)
2. Engvall, E., Perlmann, P., J. Immunol. **109**, 129-135 (1972)
3. Remington, J.S., Klein, J.O., Infectious diseases of the fetus and newborn infant. Sanders, Philadelphia, London, Toronto (1976)
4. Bidwell, D.E. et al., J. Infect. Dis. **136**, Supplement 274-278 (1977)
5. Volk, W.A. Essentials of Medical Microbiology. Second ed., J.B. Lippincott

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HSV 2 IgG

ELISA Test for the Determination of IgG Antibodies to *Herpes Simplex Virus* type 2 in Human Serum and Plasma

Package Size

[REF]	51226	96 Tests	Complete Test Kit
[IVD]			

Intended Use

HUMAN HSV 2 IgG ELISA is a colorimetric enzyme immunoassay for the qualitative (cut-off) detection of IgG class antibodies against Herpes Simplex Virus type 2 (HSV 2) in human serum and plasma (heparin, EDTA, citrate). It is used as an aid in the diagnosis of HSV 2 infection. The test can further be performed on pregnant women as part of a prenatal screening. HUMAN HSV 2 IgG is designed for manual processing as well as for automated use with ELISA analysers. For laboratory professional use only.

Principle - Classic EIA

The HUMAN HSV 2 IgG ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with recombinant gG2 protein. In the first incubation step corresponding specific antibodies (HSV 2-IgG-Ab) present in patient specimens or controls bind to the immobilized antigen at the solid phase. At the end of the incubation unbound components are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugated) is added which binds specifically to IgG class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the HSV 2-IgG-Ab concentration in the specimen.

The optical density (OD) of controls and specimens is determined by using microplate readers or automated ELISA analysers. A specimen is classified positive or negative against a controls-derived cut-off OD. The cut-off has been set in reference to in-house and external commercially available quality control samples.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) (Code HS2 G) breakable 8-well snap-off strips coated with recombinant gG2 protein	
[NC]	2.5 ml	Negative Control (green cap) ready for use Human serum Sodium azide Thimerosal	
[PC]	2.5 ml	Positive Control (red cap) ready for use Human serum Sodium azide Thimerosal	
[DIL-G]	100 ml	Dilution Buffer IgG (white cap)	
5121		ready for use, coloured green Phosphate buffer Sodium azide Thimerosal	pH 6.5 ± 0.2
[CON]	12 ml	Anti-IgG Conjugate (white cap) ready for use, coloured red Anti-human IgG (rabbit), peroxidase-conjugated Thimerosal	
[WS]20x]	50 ml	Washing Solution (white cap)	
5102		Concentrate for about 1000 ml Tris buffer Thimerosal	pH 7.2 ± 0.2
[SUB]	13 ml	Substrate Reagent (black cap)	
5103		ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin (TMB) Hydrogen peroxide	pH 3.7 ± 0.2
[STOP]	15 ml	Stop Solution (red cap)	
5104		Sulphuric acid, ready for use	0.5 mol/l
	2	Adhesive Strips	

Preservatives: Total concentration < 0.1%

General-purpose reagents [DIL-G] 5121, [WS]20x] 5102, [SUB] 5103 and [STOP] 5104 are interchangeable between HUMAN ELISA lots and kits that share the same reagent designation. For IgG tests use only IgG dilution buffer [DIL-G] 5121.

All other reagents are specific for the individual package lot and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Additional materials recommended but not supplied with the kit

Test tubes for sample dilution, micropipettes, fresh pipette tips, ELISA washer (optional), microplate reader equipped with 450 nm or with 450/620–680 nm filters, deionised water (diH_2O).

Readers and automated analysers

Validated settings for the following HUMAN ELISA devices are preinstalled or can be obtained from your local distributor. Application sheets for HUMAN instruments with analyser/assay-specific handling and performance information are accessible via: www.human.de/aps-elisa

HUMAN ELISA instrument	Instrument type	REF
HumaReader Single plus	microplate reader	18000
HumaReader HS	microplate reader	16670
Elisys Uno	automated analyser	17350
Elisys Duo	automated analyser	17200

Storage/stability

Unopened at 2…8 °C	up to the stated expiration date
Opened at 2…8 °C	60 days
[WASH] at 17…25 °C	60 days

Reagent preparation

Bring all reagents to 17...25°C before use. Reagents not in use should always be stored at 2...8 °C.

[MIC] (Code: HS2 G)

- Allow to reach 17...25°C before opening!
- Lost vacuum in the bag of the coated plate will not affect the performance of the test.
- Return unused [MIC] to the re-sealable zip-lock bag and store it with desiccant at 2...8 °C.
- Do not touch the upper rim or the bottom of the wells with fingers.

Working wash solution [WASH]

- Dilute [WS]20x] 1+19 with fresh deionised water in a suitable container, e.g. 10 ml [WS]20x] + 190 ml diH_2O = 200 ml.
- [WASH] should always be stored at 17…25°C.

Specimen

- Un-pooled and undiluted human serum and plasma with anticoagulants citrate, heparin or EDTA. Do not use specimens preserved with sodium azide.
- Do not use highly lipemic or hemolysed specimens.
- Specimens may be stored for 7 days at 2...8°C or longer at -20°C. Freeze and thaw once only**.** Thawed specimen must be homogenised.
- Particulate matter and fibrin particles may yield reactive results. Eliminate particulate matter by centrifugation or filtration.
- Incompletely coagulated sera and microbially-contaminated specimens should not be used.

Procedure

Follow the procedure exactly as described.

Procedural notes

P1: Do not mix reagents from different lot numbers within a run unless they are general purpose reagents. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of microtiter strips.

P5: Do not let the wells dry once the assay has been started.

P6: Do not reuse the coated plates.

P7: Never use the same container for conjugate and solutions.

P8: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at halfway time of the series. If more than 1 plate is used, repeat the controls for each plate.

P9: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P10: Avoid/remove air bubbles prior to incubation and reading of absorbance.

P11: Do not expose the reagents to excessive heat or sunlight during storage and test procedure.

P12: Do not use reagents without label or with damaged package.

P13: Always firmly close vials with the proper caps after use.

P14: Remove only reagents required for a run from stock solutions since they could come into contact with other contaminating solutions like patient specimens etc.

P15: Always store stock solutions at 2...8 °C when not in use; do not freeze the reagents.

P16: Do not run the test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision. The use of an automatic washer is strongly recommended.

W1: Remove the adhesive protective film from [MIC] if applied and transfer the contents of all wells into 5% sodium hypochlorite solution or another disinfectant.

W2: Add [WASH] to each well at the volume specified in the Pipetting scheme. Aspirate after a soak time of 30 s. Ensure that no liquid is left in the wells (use double aspiration in the final step where possible). Repeat this step as specified in the Pipetting scheme.

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper. Residual volume lower than 10 µl is not critical for the assay.

Pipetting Scheme

Sample Preparation:

Dilute patient's sera 1+100 with [DIL-G] 5121: 10 µl serum + 1 ml [DIL-G] 5121 Mix thoroughly (15s).

Diluted samples can be stored up to 48 h at 2...8°C before testing.

[NC], [PC] are ready for use, rock gently but thoroughly before use.

Step 1	*Well [µl]*			
	A1 Blank	B1/C1 [NC]	D1/E1 [PC]	F1... Sample
[NC] in duplicate	--	100	--	--
[PC] in duplicate	--	--	100	--
Diluted samples	--	--	--	100
Mix carefully				
Seal [MIC] with Adhesive Strips				
Incubate 30 min at 17...25°C				
Wash as described: W1, **4x** W2, W3				
[WASH]	350	350	350	350
Step 2				
[CON]	--	100	100	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash as described: W1, **5x** W2, W3				
[WASH]	350	350	350	350
Step 3				
[SUB] 5103	100	100	100	100
Incubate 15 min at 17...25°C in the dark (see P9)				
[STOP] 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.				
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction. Use a reference wavelength of 620-680 nm if available.				

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] in wells B1 and C1 (MNC), [PC] in wells D1 and E1 (MPC) are calculated according to:

$$MNC = \frac{A_{450}(B1) + A_{450}(C1)}{2}; \quad MPC = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

Cut-off value COV = MNC + (0.1 x MPC)

The test run is valid if the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ 0.250
3. MPC ≥ 0.750
4. MPC: MNC ≥ 5

If invalid, re-run the test paying careful attention to the procedure as described above.

Interpretation of Results

Result	Interpretation
A_{450} (patient) ≥ COV + 15%	anti-HSV 2-IgG-Ab-positive
A_{450} (patient) < COV -15%	anti-HSV 2-IgG-Ab-negative
A_{450} (patient) ≥ COV -15% and A_{450} (patient) < COV + 15%	equivocal: retest (patient monitoring is recommended in case of a reoccurring equivocal result to exclude unspecific reactions or cross reactions)

Due to physiological and analytical variations a patient result lying 15% above or below the calculated cut-off value is classified equivocal. It is recommended to re-assay such a sample in parallel with a fresh sample taken 7 to 14 days later. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the patient history and additional investigations. Repeatedly equivocal samples may be subjected to a confirmatory test.

For accurate typing it is recommended to isolate the virus and identify the antigen with type-specific antisera. Due to the reactivation of latent infections with other viruses related to the Herpes viridae family, positive HSV 2 IgG results may occur in sera from patients with such infections. The possibility of infections with other members of the Herpes viridae family should therefore be investigated before inter-pretation of results.

Performance Characteristics

Calibration/Traceability

No international standard or other certified reference material for the biological activity of human IgG antibodies to *Herpes Simplex Virus* type 2 is available.

Precision

Precision of HUMAN HSV 2 IgG was evaluated by using 3 serum samples with grey zone (+/-), low positive (LP) and high positive (HP) status. Assay precision was determined by testing each sample in duplicate, 2 runs per day, for 20 days, using 1 lot.

	Mean [S/CO]	Repeatability	Within-lab precision
	(N=80)	SD	CV	SD	CV
Sample 1 (+/-)	1.4	0.11	8.3%	0.21	15.8%
Sample 2 (LP)	2.6	0.11	4.2%	0.31	12.0%
Sample 3 (HP)	4.6	0.13	2.8%	0.56	12.1%

Interference

Interfering substances were added to a known sample. No interference was detected for the respective substances up to following concentrations: bilirubin 40 mg/dl, haemoglobin 1000 mg/dl, lipids 2500 mg/dl.

Cross-reactivity

The assay has been tested and does not show cross-reactivity with rheumatoid factor and Anti-nuclear antibodies (ANA). Rare cross-reactivity occurred with HSV 1 IgG in 1 of 28 cases. Other members of the herpes virus family (EBV IgG, CMV IgG, VZV IgG) can in rare cases react positively in the HUMAN HSV 2 IgG ELISA.

Sensitivity and specificity

Diagnostic performance of HUMAN HSV 2 IgG has been determined with 113 samples using discrepant analysis with Novatec HSV 2 IgG ELISA as reference assay.

			Reference (resolved)	
			+	-
		113	43	70
HUMAN HSV 2 IgG	+	49	43	6
	-	64	0	64

Overall agreement:	94.7%
Sensitivity (agreement positive results):	100.0%
Specificity (agreement negative results):	91.4%

Limitations

1. All protocols for automated analysers must be fully validated prior to usage.
2. Since the enzyme reaction is very sensitive to metal ions, do not allow any metal element to come into contact with conjugate or substrate solutions. Upon development of automated applications, the compatibility of any metal tips used for dispensing has to be addressed in the validation.
3. As with all diagnostic tests, the results should be interpreted with due consideration of other laboratory findings and of the clinical status of the patient. A negative result does not preclude the possibility of exposure to or infection with HSV type 2.

Safety notes

~~All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious.~~

~~[SUB] Danger~~

~~H360D May damage the unborn child.~~

[STOP] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[NC] [PC] [DIL-G] [CON] [WS]20x] [SUB] [STOP]

P234 Keep only in original packaging.

P260 Do not breath dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/ national/ international regulations.

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious.

References

1. Engvall, E., Perlmann, P., Immunochemistry 8, 871-874 (1971)
2. Engvall, E., Perlmann, P., J. Immunol. 109, 129-135 (1972)
3. Remington, J.S., Klein, J.O., Infectious diseases of the fetus and newborn infant. Sanders, Philadelphia, London, Toronto (1976)
4. Bidwell, D.E. *et al*., J. Infect. Dis. 136, Supplement 274-278 (1977)
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Toxo IgG

ELISA Test for the Detection of IgG Antibodies to *Toxoplasma gondii* in Human Serum

Package Size

[REF]	51209	96 Tests	Complete Test Kit
[IVD]			

Intended Use

TOXO IgG ELISA is intended for the detection of Immunoglobulin G (IgG) class antibodies to *Toxoplasma gondii* in human serum.

Toxoplasma infects nearly all mammalians and birds. It is the most widely distributed of all intracellular parasites. Humans become infected through contamination with feces or uncooked meat, or through direct inoculation via blood transfusions or congenital transmission.

Pregnant women who acquire toxoplasmosis during the first trimester have a 25% risk of fetal transmission resulting in spontaneous abortions, stillborns, or severe disease. 65% of infants born to women infected during the third trimester have subclinical infection with ultimately 85% developing chorioretinitis or neurological sequelae.

Principle - Classic EIA -

HUMAN TOXO IgG ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid are coated with *Toxoplasma* antigens (TOXO-Ag) prepared from sonicated whole *Toxoplasma gondii* parasites (Tachyzoites). In the first incubation step corresponding specific antibodies (TOXO-IgG-Ab) present in patient specimens or controls bind to the antigens at the solid phase. At the end of the incubation unbound components are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugated) is added which binds specifically to IgG class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the TOXO-IgG-Ab concentration in the specimen.

Absorbance of controls and specimens is determined by using ELISA microplate readers or automated ELISA systems at 450 nm or 450 and 630–690 nm. HUMAN ELISA are compatible with both manual and automated applications (see “Readers and Automated Analyzers” and “Notes”).

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) (Code TOX G) 8-well snap-off strips coated with sonicated *Toxoplasma gondii* antigen	
[NC]	2.5 ml	TOXO IgG Negative Control (green cap) ready for use, human	
[CC]	2.5 ml	TOXO IgG Cut-Off Control (white cap) ready for use, human	1.6 IU/ml
[PCL]	2.5 ml	TOXO IgG Positive Control Low (red cap) ready for use, human	10 IU/ml
[PCM]	2.5 ml	TOXO IgG Positive Control Medium (red cap) ready for use, human	33 IU/ml
[PCH]	2.5 ml	TOXO IgG Positive Control High (red cap) ready for use, human	65 IU/ml
		[CC], [PCL], [PCM], [PCH]: Calibrated against the WHO 1st International Standard for human anti-Toxoplasma IgG (NIBSC code 01/600).	
[DIL-G] 5121	100 ml	Dilution Buffer IgG (white cap) Ready for use, coloured green Phosphate buffer NaCl Albumin	 pH 6.5 ± 0.2 10 mmol/l 8 g/l 10 g/l
[CON]	12 ml	Anti-IgG Conjugate (white cap) Ready for use, coloured red Anti-human IgG (rabbit), peroxidase-conjugated	
[WS]20x] 5102	50 ml	Washing Solution (white cap) Concentrate for about 1000 ml Tris buffer NaCl	 pH 7.2 ± 0.2 10 mmol/l 8 g/l
[SUB] 5103	13 ml	Substrate Reagent (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin (TMB) Hydrogen peroxide	 pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l
[STOP] 5104	15 ml	Stop Solution (red cap) Sulphuric acid, ready for use	 0.5 mol/l
	2	Adhesive Strips	

Preservatives: Total concentration < 0.1%

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/630–690 nm filters, deionised water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

[MIC] (Code: TOX G)

- sealed in an aluminium bag with a desiccant.
- must be at room temperature before opening,
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C.

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The general purpose reagents [DIL-G] 5121, [WS]20x] 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits. For IgG tests use only IgG dilution buffer [DIL-G] 5121.

All other reagents are specific for the individual package lot and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution **[WASH]**

- dilute [WS]20x] 5102 1+19 with fresh deionised water, e.g. 50 ml [WS]20x] 5102 + 950 ml = 1000 ml.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of Microtiter Strips.

P5: Run duplicates for controls. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P9: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add [WASH] to each well, aspirate off after 30 sec. soak time and repeat washing 3 resp. 4 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.
Sample Preparation:

Dilute the patient's sera 1+100 with [DIL-G] 5121, e.g. 10 µl serum + 1 ml [DIL-G] 5121, mix thoroughly.

Diluted samples can be stored up to 48 h at 2...8°C before testing.

Controls are ready for use.

Step 1	*Well [µl]*			
	A1 Blank	B1/C1 [NC]	D1/C2 [PC]	D2... Sample
[NC] in duplicate	--	100	--	--
[CC] in duplicate, D1/E1	--	--	100	--
[PCL] in duplicate, F1/G1	--	--	100	--
[PCM] in duplicate, H1/A2	--	--	100	--
[PCH] in duplicate, B2/C2	--	--	100	--
Diluted samples	--	--	--	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 2				
[CON]	--	100	100	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 3				
[SUB] 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
[STOP] 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.				
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

Readers and Automated Analyzers

Validated settings for HUMAN ELISA microplate readers (HumaReader) or automated HUMAN ELISA analyzers (ELISYS line) are preinstalled or can be obtained from your local distributor. Application sheets for Human instruments with analyzer/assay specific handling and performance information are accessible via: www.human.de/aps-elisa.

For automated analysers other than those provided by HUMAN, follow the Pipetting Scheme section and ensure all requirements described in the Procedural Notes section are followed. All protocols for automated analyzers must be fully validated before usage.

Results for patient samples are obtained either by comparison with a cut-off control or in IU/ml by quantitative estimation using a calibration curve constructed with the help of the cut-off and 3 positive controls.

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] (MNC), of [CC] (MCC) and of [PCL], [PCM], [PCH] (MPCL, MPCM, MPCH) are calculated according to the following example:

$$MCC = \frac{A_{450}\,(D1) + A_{450}\,(E1)}{2}$$

The test run may be considered valid if the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ MCC < MPCL < MPCM < MPCH
3. MPCM ≥ 0.750
4. MPCM / MNC ≥ 5

Interpretation of Results

A_{450} (patient) ≥ MCC + 15%: anti-TOXO-IgG-Ab-positive

A_{450} (patient) < MCC -15%: anti-TOXO-IgG-Ab-negative

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Quantitative Estimation of anti-Toxoplasma IgG in Patient Samples

For a quantitative estimate of levels of anti-Toxoplasma IgG in positive specimens in IU/ml, MCC, MPCL, MPCM and MPCH (ordinate) are plotted in a graph versus their corresponding anti-Toxo IgG concentrations of 1.6, 10, 33, and 65 IU/ml (abscissa). A calibration curve is made by connecting point to point with straight lines. Levels of anti-Toxo IgG in patient sera are read off the calibration curve using their individual A_{450}.

Patient sera with A_{450} greater than the [PCH] (65 IU/ml) should be further diluted with [DIL-G] and reassayed before estimating antibody levels.

The clinical significance of changes in *Toxoplasma gondii* specific IgG levels must be interpreted with care.

Performance Characteristics

The assay has been standardised against the WHO 1st IS for human anti-Toxoplasma IgG, NIBSC code 01/600.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-toxog.pdf or

www.human-de.com/data/gb/vr/el-toxog.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Notes

1. All protocols for automated analyzers must be fully validated prior usage.
2. The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

Safety Notes

[STOP] Warning

~~. Hazard statements~~

H315 Causes skin irritation.

H319 Causes serious eye irritation.

~~[SUB] **Danger**~~

~~. Hazard statements~~

~~H360D May damage the unborn child.~~

~~. Precautionary statements~~

[NC] [CC] [PCL] [PCM] [PCH] [DIL-G] [CON] [WS20x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative~~.

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

Literature

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4. Bidwell, D.E. et al., J. Infect. Dis. 136, Supplement 274-278 (1977)
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Toxo IgM µ-capture

Immunocapture ELISA for the Determination of IgM Antibodies to *Toxoplasma gondii* in Human Serum and Plasma

Package Size

[REF]	51119	96 Tests	Complete Test Kit

[IVD]

Intended Purpose

HUMAN Toxo IgM µ-capture ELISA is a colorimetric enzyme immunoassay for the qualitative determination of IgM-class antibodies to *Toxoplasma gondii* in human serum or plasma. It is used as an aid in the diagnosis of acute *Toxoplasma* infection in symptomatic individuals, for screening of pregnant women for a recent infection and for confirmation of suspected congenital toxoplasmosis in newborns. HUMAN Toxo IgM µ-capture ELISA is designed for manual use or for processing with automated ELISA analysers. For laboratory professional use only.

Clinical Significance

Toxoplasma gondii infects nearly all mammals and birds. It is the most widely distributed of all intracellular parasites. Human infections primarily arise upon ingesting undercooked or raw meat containing viable tissue cysts, or by ingesting food or water contaminated with *T. gondii* oocysts, typically by faeces of infected cats. Direct infections follow via blood transfusion or congenital transmission.

Pregnant women who acquire toxoplasmosis in the 1st trimester have a 25% risk of fetal transmission resulting in spontaneous abortions, stillbirths, or severe disease. 65% of infants born to women infected during the 3rd trimester have subclinical infection with ultimately 85% developing chorioretinitis or neurological sequelae.

Toxoplasmosis in humans is typically diagnosed with the aid of serologic testing for antibodies against the parasite (anti-Toxo). A test specific for the IgM-class of anti-Toxo can help to identify an acute or recent infection, which can be of particular importance to pregnant women.

Principle - µ-Capture-Assay and Direct IgM Detection

µ-capture ELISA for direct detection of specific IgM antibodies makes use of mouse monoclonal anti-human-IgM antibodies coated on the surface of microtiter wells. In step 1, any IgM-class antibodies present in the specimen bind to the immobilised capture antibodies. In step 2, unbound specimen components are washed out and the wells are incubated with a conjugate of toxoplasma antigens and the horseradish peroxidise (HRP) enzyme. The conjugate binds to those captured IgM that are specific for the toxoplasma antigens. In step 3, unbound conjugate is removed and TMB, a chromogenic substrate of the HRP enzyme, is added. The HRP moiety of bound conjugate converts colourless TMB to a water-soluble blue reaction product. A pH drop stops the reaction and turns the product yellow. Coloration intensity is proportional to the concentration of anti-Toxo IgM in the specimen.

The optical density (OD) of controls and specimens is determined by using microplate readers or automated ELISA analysers. A specimen is classified reactive or non-reactive for anti-Toxo IgM against a controls-derived cut-off OD. The cut-off has been set in reference to in-house and external commercially available quality control samples.

Reagents and Contents

[MIC]	12	Microtiter strips (in strip holder) breakable 8-well strips coated with specific monoclonal anti-human-IgM	
[NC]	2.5 ml	Negative control (green cap) diluted human serum negative for anti-Toxo IgM, ready for use, dyed green	
[PCL]	2.5 ml	Positive control, low (red cap) diluted human serum positive for anti-Toxo IgM, ready for use, dyed green	
[PCH]	2.5 ml	Positive control, high (red cap) diluted human serum positive for anti-Toxo IgM, ready for use, dyed green	
[DIL-M] 5112	100 ml	Sample diluent (blue cap) ready for use, dyed green phosphate buffer	pH 6.5 ± 0.1
[CON]	15 ml	Enzyme conjugate (white cap) ready for use, dyed orange-red Toxoplasma antigen, HRP-conjugated	
[WS]20x] 5102	50 ml	Wash solution (white cap) concentrate for 1000 ml TRIS buffer, NaCl	pH 7.2 ± 0.1
[SUB] 5103	13 ml	Substrate reagent (black cap) ready for use, colourless 3,3',5,5'-tetramethylbenzidine (TMB) hydrogen peroxide	
[STOP] 5104	15 ml	Stop solution (red cap) sulphuric acid, ready for use	0.5 mol/l
	1	Adhesive strips	

Preservatives: total concentration < 0.1%

General-purpose reagents [DIL-M] 5112, [WS]20x] 5102, [SUB] 5103 and [STOP] 5104 are interchangeable between HUMAN ELISA lots and kits that share the same reagent designation.

Additional Materials Required but not Supplied with the Kit

Test tubes for sample dilution, micropipettes, fresh pipette tips, ELISA washer (optional), microplate reader equipped with 450 nm or with 450/620–690 nm filters, deionised water (diH_2O).

Readers and Automated Analysers

Validated settings for the following HUMAN ELISA instruments are preinstalled or can be obtained from your local distributor. Application sheets for HUMAN instruments with analyser/assay-specific handling and performance information are accessible via: www.human.de/aps-elisa

HUMAN ELISA Instrument	Instrument type	REF
HumaReader Single plus	microplate reader	18000
HumaReader HS	microplate reader	16670
Elisys Uno	automated analyser	17350
Elisys Duo	automated analyser	17200

For automated analysers other than those provided by HUMAN follow the Pipetting scheme section and ensure all requirements described in the Procedural notes section have been satisfied. All protocols for automated analysers must be fully validated prior to use.

Storage/Stability

Unopened at 2…8 °C	up to the stated expiration date
Opened at 2…8 °C	60 days
[WASH] at 17…25 °C	60 days

Reagent Preparation

Bring all reagents to 17...25 °C before use. Reagents not in use should always be stored at 2...8 °C.

[MIC]

- Allow to reach 17…25 °C before opening!
- Lost vacuum in the bag of the coated plate will not affect the performance of the test.
- Return unused [MIC] to the re-sealable zip-lock bag and store it with desiccant at 2...8 °C.
- Do not touch the upper rim or the bottom of the wells with fingers.

Working wash solution [WASH]

- Dilute [WS]20x] 1+19 with fresh deionised water in a suitable container, e.g. 10 ml [WS]20x] + 190 ml diH_2O = 200 ml.

Specimens

- Un-pooled and undiluted human serum or plasma (anticoagulants: EDTA, heparin or citrate). Do not use specimens preserved with sodium azide.
- Avoid haemolysis by separating as soon as possible whole blood specimens. Do not use highly lipemic, haemolysed or icteric specimens. Remove any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen before use. Do not use heated specimens.
- Specimens may be stored for 7 days at 2...8 °C or for longer at -20 °C. Freeze and thaw once only. Thawed specimen must be homogenised.
- Particulate matter and fibrin particles may yield reactive results. Avoid pipetting particulate matter.
- Incompletely coagulated sera and microbially-contaminated specimens should not be used.

Procedure

Follow the procedure exactly as described!

Procedural Notes

P1: Do not mix reagents from different lot numbers within a run unless they are general purpose reagents. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.
P2: Each sample, control and reagent must be pipetted with a single fresh tip.
P3: Do not use reagents that could be contaminated or look or smell different than usual.
P4: Record specimens and controls carefully on the spread sheet supplied with the kit.
P5: [MIC] - select the required number of microtitre strips.
P6: Do not let the wells dry once the assay has been started.
P7: Do not reuse the coated plates.
P8: Never use the same container for conjugate and solutions.
P9: Always pipette controls, samples and reagents in the same order and timing according to the assay procedure and without interruptions to minimise reaction time differences between wells. This is important for reproducible results.
P10: Avoid/remove air bubbles prior to incubation and reading of absorbance.
P11: Do not expose the reagents to excessive heat or sunlight during storage and test procedure.
P12: Do not use reagents without label or with damaged package.
P13: Always firmly close vials with the proper caps after use.
P14: Remove only reagents required for a run from stock solutions since they could come into contact with other contaminating solutions like patient specimens etc.
P15: Always store stock solutions at 2...8 °C when not in use; do not freeze the reagents.
P16: Do not run the test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals.
P17: Since the enzyme reaction is very sensitive to metal ions, do not allow any metal element to come into contact with conjugate or substrate solutions. Upon development of automated applications, the compatibility of any metal tips used for dispensing has to be addressed in the validation.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision. The use of an automatic washer is strongly recommended.

W1: Remove the adhesive protective film from [MIC] if applied and transfer the contents of all wells into 5% sodium hypochlorite solution or another disinfectant.
W2: Add [WASH] to each well at the volume specified in the Pipetting scheme. Aspirate after a soak time of 30 s. Ensure that no liquid is left in the wells (use double

aspiration in the final step where possible). Repeat this step as specified in the Pipetting scheme.

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Sample preparation:
Dilute patient's sera 1+100 with [DIL-M]: 10 µl serum + 1 ml [DIL-M]. Mix thoroughly (15 s).
Diluted samples must be used on the day of dilution.
[NC], [PCL] and [PCH] are ready for use, rock gently but thoroughly before use.

Step 1	*Well [µl]*				
	A1-B1 Blank	C1-D1 [NC]	E1-F1 [PCL]	G1-H1 [PCH]	A2... Sample
[DIL-M] in duplicate	100				
[NC] in duplicate	--	100	--	--	--
[PCL] in duplicate	--	--	100		--
[PCH] in duplicate	--	--	--	100	--
Diluted samples in duplicate	--	--	--	--	100
Mix carefully					
Seal [MIC] with adhesive strips					
Incubate for 60 min at 17…25°C					
Wash as described: W1, **4x** W2, W3					
[WASH]	350	350	350	350	350
Step 2					
[CON]	100	100	100	100	100
Seal [MIC] with adhesive strips					
Incubate for 30 min at 17…25°C					
Wash as described: W1, **5x** W2, W3					
[WASH]	350	350	350	350	350
Step 3					
[SUB]	100	100	100	100	100
Incubate for 30 min at 17…25°C in the dark					
[STOP]	100	100	100	100	100
Mix carefully					
Measure the optical density at 450 nm 2-10 min after terminating the reaction. Use a reference wavelength of 620-690 nm if available.					

Calculation of Control Values and Cut-Off

Calculate mean blank OD (MBlank) as shown below. Subtract MBlank from the OD values of the controls (C1-H1) and samples (A2, ...) and calculate mean absorbance values of [NC] in wells C1 and D1 (MNC), [PCL] in wells E1 and F1 (MPCL) and [PCH] in wells G1 and H1 (MPCH) according to:

$$\text{MBlank} = \frac{A_{450}\,(A1) + A_{450}\,(B1)}{2} \qquad \text{MNC} = \frac{A_{450}\,(C1) + A_{450}\,(D1)}{2}$$

$$\text{MPCL} = \frac{A_{450}\,(E1) + A_{450}\,(F1)}{2} \qquad \text{MPCH} = \frac{A_{450}\,(G1) + A_{450}\,(H1)}{2}$$

Cut-off value COV = MNC + 0.2 x MPCL

The test run is valid if the following criteria are met:

1. MNC < 0.05
2. MPCL ≥ 0.20
3. MPCH > MPCL
4. MPCL / COV ≥ 3.0

If invalid, re-run the test paying careful attention to the procedure as described above.

Interpretation of Results

A specimen is classified reactive, non-reactive or equivocal for anti-Toxo IgM by comparing the mean OD measured for the specimen ($MOD_{specimen}$) to the COV. The specimen is assigned no value in IU/L of biological activity of human anti-Toxo IgM.

Result	Interpretation
$MOD_{specimen}$ < COV - 10%	nonreactive for anti-Toxo-IgM
$MOD_{specimen}$ ≥ COV + 10%	reactive for anti-Toxo-IgM
$MOD_{specimen}$ ≥ COV - 10% and $MOD_{specimen}$ < COV + 10%	equivocal: retest (patient monitoring is recommended in case of a reoccurring equivocal result to exclude unspecific reactions or cross reactions)

Due to physiological and analytical variations, a patient result lying 10% above or below the calculated cut-off value is classified equivocal. It is recommended to re-assay such a sample in parallel with a fresh sample taken 7 to 14 days later. The trend between the specific antibody levels should be used for interpretation.

Performance Characteristics

Calibration/Traceability

No international standard or other certified reference material for the biological activity of human IgM antibodies to *T. gondii* is available.

Precision

Precision of HUMAN Toxo IgM µ-capture was evaluated by means of a modified CLSI EP5 protocol using four controls with different levels of anti-Toxo IgM. Assay precision was determined by testing each sample in duplicate, 2 runs per day, for 20 days.

Sample	Mean (S/Co)	Repeatability (%CV)	Within-lab variability (%CV)	Lot-to-lot variability
1	2.9	1.7%	5.4%	15.7%
2	13.8	1.9%	5.2%	15.7%
3	8.0	1.5%	5.0%	13.4%
4	2.0	3.1%	6.0%	18.3%

Interference

Interfering substances were added to a known sample. No interference was detected for the respective substances up to following concentrations: bilirubin 40 mg/dl, haemoglobin 1 g/dl, triglycerides 1 g/dl, parenteral nutritional lipid supplement 1 g/dl.

Sensitivity and Specificity

Diagnostic performance of HUMAN Toxo IgM µ-capture has been determined on a pool of 206 samples using discrepant analysis with a competitor CE-marked anti-Toxo IgM µ-capture ELISA as the primary reference and a CE-marked anti-Toxo IgM CLIA as the resolver assay. Borderline samples and samples where the primary and the resolver reference assays disagreed were excluded from the evaluation.

			Reference (resolved)	
			+	-
		195	50	145
HUMAN Toxo IgM µ-capture	+	49	49	0
	–	146	1	145

Overall agreement:	99.5% (95% CI: 97.2% to 99.9%)
Sensitivity (agreement positive results):	98.0% (95% CI: 89.5% to 99.6%)
Specificity (agreement negative results):	100.0% (95% CI: 97.4% to 100.0%)

Limitations

- Specimens from patients infected with EBV can in some cases react positively in the HUMAN Toxo IgM µ-capture ELISA. Positive reactions of specimens containing anti-Plasmodium IgM cannot be excluded. The assay has been tested for and does not show cross-reactivity with rheumatoid factor IgM.
- As with all diagnostic tests, the results should be interpreted with due consideration of other laboratory findings and of the clinical status of the patient. A positive result for anti-Toxo IgM may have to be confirmed by a national reference laboratory in compliance with national guidelines. A negative result does not preclude the possibility of exposure to or infection with *T. gondii*.

Safety Notes

~~All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.~~

~~[SUB] Danger~~

~~H360D May damage the unborn child.~~

[STOP] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[NC] [PCL] [PCH] [DIL-M] [CON] [WS]20x] [SUB] [STOP]

P234 Keep only in original packaging.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/ national/ international regulations.

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious.

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EL-ToxMµ INF 51119 GB 02-2022-008

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CA 125 Ag

ELISA for the Quantitative Determination of Cancer Antigen 125 (CA 125 Ag) in Human Serum

Package Size

[REF]	52050	96 Tests	Complete Test Kit

[IVD]

Intended Use

Cancer Antigen 125 (CA 125) has been characterised as a high molecular weight glycoprotein of about 200 kDa. It is the most important tumour marker associated with ovarian cancer.

High levels of CA 125 occur in patients suffering from ovarian cancer, while elevated levels are also observed in a range of benign and other malignant diseases. Although specificity and sensitivity of CA 125 is limited, particularly for early diagnosis, it is the first marker for monitoring of therapy and patient progress. Higher tumour stages are associated with higher CA 125 levels.

Principle

The HUMAN CA 125 Ag ELISA intended for professional use, is an ELISA for direct antigen detection including high affinity and specificity antibodies (enzyme labelled and biotinylated) with different and distinct epitope recognition.

The assay makes use of the extremely high affinity of the system Biotin-Streptavidin. Streptavidin has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls, enzyme conjugate (peroxidase-labelled anti-CA 125, monoclonal) and a second biotinylated monoclonal anti-CA 125 are mixed to form the sandwich complex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin. At the end of the incubation excess enzyme conjugate and monoclonal antibodies are washed out. Substrate TMB is added (step 2) and the resulting colour, which turns into yellow after stopping the reaction with the stop solution, is measured photometrically. The intensity of colour is directly proportional to the CA 125 concentration in the sample.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) breakable 8-well strips coated with streptavidin	
[CAL]	A - F 6x2 ml	Calibrators (imprinted, white cap) ready for use, human serum, yellowish CA 125 level: (A) 0 U/ml, (B) 15 U/ml, (C) 50 U/ml, (D) 100 U/ml, (E) 200 U/ml, (F) 400 U/ml	
[CON]	13 ml	Enzyme Conjugate (white cap) Ready for use, coloured red anti- CA 125 Ag (mouse, monoclonal) HRP labelled and monoclonal anti- CA 125 Ag (mouse) biotinylated	
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for about 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidin (TMB) Hydrogen peroxide Sodium-Acetate buffer	<0.25 g/l 0.03 ml/l
[STOP]	8 ml	Stop Solution (red cap) Sulphuric acid, ready for use	0.5 mol/l
	1	Adhesive Strips	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with desiccant
- Must be at room temperature before opening
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic, hemolysed specimen or specimen containing sodium azide.

Specimens may be stored for 5 days at 2...8°C or up to 30 days at -20°C, Freeze and thaw twice only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of Microtiter Strips.

P5: Run duplicates or triplicates for positive resp. negative control. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents and add [WASH] to each well, aspirate off after 40-60 sec. soak time and repeat washing 2 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 40-60 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	F2... Specimen
[CAL] A-F in duplicate	25	--
Samples, controls in duplicate	--	25
[CON]	100	100
Mix and cover [MIC] with Adhesive Strips		
Incubate 60 min. at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min. at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 620-630 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line) at 450 nm. The concentrations for patient samples are obtained by means of a calibration curve which is established from the calibrators supplied with the kit

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] F ≥1.3.
- The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points results in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

The level of CA 125 cannot be used as absolute evidence for the presence or absence of malignant disease due to low clinical sensitivity and specificity of this tumour marker.

Determination of CA 125 is intended for monitoring of therapy and patient progress. The results should only be used in conjunction with other clinical investigations and procedures in the diagnosis and prognosis of disease.

The laboratory finding must always include a statement on the CA 125 method used due to potential variations in the result depending on the test procedure used. Therefore CA 125 values determined with different test procedures cannot be directly compared with one another. Change of assay procedure during monitoring of patients must be confirmed by parallel measurements with both procedures.

Expected values

CA 125 is elevated in women with malignant diseases, but may also be elevated in about 1% normal, healthy women, as well as in about 5% women with benign ovarian diseases and non-neoplastic conditions (including first trimester of pregnancy menstruation, endometriosis, hepatic diseases).

Expected values for the CA 125 Ag ELISA:

Healthy and non-pregnant donors: ≤ 35 U/ml

Each laboratory should establish its own expected values to its own patient population utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-ca125.pdf or

www.human-de.com/data/gb/vr/el-ca125.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

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EL-CA125 INF 5205001 GB 07-2015-10M

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CA 15-3 Ag

ELISA for the Quantitative Determination of Cancer Antigen 15-3 (CA 15-3 Ag) in Human Serum

Package Size

REF	52080	96 Tests	Complete Test Kit
IVD			

Intended Use

Cancer Antigen 15-3 has been characterised as sialylated high molecular weight glycoprotein of about 400 kDa. Serum level of CA 15-3 provides information about the clinical stage of the breast disease.

CA 15-3 is more sensitive than CEA in early detection of breast cancer, however recent reports indicate a clear increase in diagnostic sensitivity for combined use of both tumour markers.

High levels of CA 15-3 occur in patients suffering from breast cancer, while elevated levels are also observed in a range of benign and malignant diseases (see Expected Values). Although specificity and sensitivity of CA 15-3 Ag is limited, it is the first marker for diagnosis of breast cancer and monitoring of therapy and patient progress. Higher tumour stages are associated with higher CA 15-3 levels.

Principle – Direct Antigen EIA -

The HUMAN CA 15-3 Ag ELISA, intended for professional use, is an ELISA for direct antigen detection including high affinity and specificity antibodies (enzyme labelled and biotinylated) with different and distinct epitope recognition. The assay makes use of the extremely high affinity of the system Biotin-Streptavidin. Streptavidin has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and Conjugate B (monoclonal, biotinylated antibody) are mixed to form an antibody-antigen complex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin.

After removing and washing out of unbound components Conjugate E (enzyme-labelled antibody) is added (step 2). The enzyme conjugate binds to the immobilised antibody-antigen complex forming the final sandwich complex. After incubation excess conjugate is washed out and TMB Substrate added (step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the CA 15-3 concentration in the specimen.

Reagents and Contents

MIC	12	**Microtiter Strips** (in strip holder) breakable 8-well strips coated with streptavidin	
CAL	A - F 6x2 ml	**Calibrators** (imprinted, white cap) ready for use, human CA 15-3 level: (A) 0 U/ml, (B) 10 U/ml, (C) 40 U/ml, (D) 100 U/ml, (E) 200 U/ml, (F) 400 U/ml	
DIL	50 ml	**Diluent** (transparent cap) Tris buffered saline BSA	 1 g/l
CON-B	13 ml	**Conjugate B (biotin)** (white cap) Ready for use, coloured green monoclonal anti-CA 15-3 Ag antibody (mouse) biotinylated	
CON-E	13 ml	**Conjugate E (enzyme)** (white cap) Ready for use, coloured yellow monoclonal anti-CA 15-3 Ag antibody (mouse) HRP labelled	
WS 50x	20 ml	**Wash Solution** (black cap) Concentrate for about 1000 ml Tris buffered saline	 250 mmol/l
SUB	13.5 ml	**Substrate Reagent** (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	 < 0.5 g/l 0.03 mol/l
STOP	8 ml	**Stop Solution** (red cap) Sulphuric acid, ready for use	 0.5 mol/l
	2	**Adhesive Strips**	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and CAL should be handled as potentially infectious. CAL have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found to be non-reactive. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or CAL should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

MIC

- Sealed in an aluminium bag with desiccant
- Must be at **room temperature** before opening
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to **room temperature** (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution WASH

- Dilute WS 50x to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: **up to 60 days at 15...25°C.**

Specimen

Serum

Do not use highly lipemic, hemolysed specimen or specimen containing sodium azide.

Specimens may be stored for 5 days at 2...8°C or up to 30 days at -20°C. **Freeze and thaw twice only**. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: MIC - select the required number of Microtiter Strips.

P5: **Run duplicates** of CAL, controls and specimens. Pipette them **on the bottom** in the microwells.

P6: **Always add reagents in the same order and timing to minimise reaction time differences between wells.** This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: SUB – incubate in the dark. SUB initiates a kinetic reaction, which is terminated by STOP.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents and add WASH to each well, aspirate off after 40-60 sec. soak time and repeat washing 2 times.

W2: In case of automatic washers fill and prime with WASH. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 40-60 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Sample Preparation: Dilute patient's sera and controls 1 + 20 with DIL, e.g. 25 µl serum + 500 µl DIL, mix thoroughly. Diluted samples can be stored up to 4 h at 2...8°C before testing. CAL A-F are ready for use.		
Step 1	***Well [µl]***	
	A1...D2 Calibrators	**E2...** Specimen
CAL A-F in duplicate	25	--
Diluted samples, controls in duplicate	--	25
CON-B	100	100
Mix 20 – 30 sec and cover MIC with Adhesive Strips		
Incubate 60 min. at 20...25°C		
Wash 3 times as described (see W1 - W3)		
WASH	350	350
Step 2		
CON-E	100	100
Do not shake MIC after CON-E addition		
MIC cover with Adhesive Strips		
Incubate 60 min. at 20...25°C		
Wash 3 times as described (see W1 - W3)		
WASH	350	350
Step 3		
SUB	100	100
Do not shake MIC after SUB addition		
Incubate 20 min. at 20...25°C (see P8)		
STOP	50	50
Mix carefully 15 – 20 sec		
Measure the absorbance at **450 nm** as soon as possible or **within 30 min.** after terminating of the reaction, using a reference wavelength of 620-630 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate reader or automated ELISA system (like HUMAN's HumaReader or ELISYS line) at 450 nm. The concentrations for patient samples are obtained by means of a calibration curve which is established from the calibrators supplied with the kit. Human ELISA are compatible with both manual and automated applications. Settings for applications of the HUMAN ELISA are preinstalled on the particular HUMAN instrument and may differ from the manual method.

Validation of the Test

The test results are valid, if the following criteria are met:

– The mean absorbance (OD) of CAL F ≥ 1.3.

– The difference between the duplicates of CAL F does not exceed 10%.

Calculation

Plot measured absorbances against CAL concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

The level of CA 15-3 cannot be used as absolute evidence for the presence or absence of malignant disease due to low clinical sensitivity and specificity of this tumour marker.

Determination of CA 15-3 is intended for monitoring of therapy and patient progress. The results should only be used in conjunction with other clinical investigations and procedures in the diagnosis and prognosis of disease.

The laboratory finding must always include a statement on the CA 15-3 method used due to potential variations in the result depending on the test procedure used. Therefore CA 15-3 values determined with different test procedures cannot be directly compared with one another. Change of assay procedure during monitoring of patients must be confirmed by parallel measurements with both procedures.

Expected values

CA 15-3 is elevated in malignant, but also in some benign diseases like benign breast diseases, hepatic, pancreatic or rheumatic diseases, and tuberculosis.

Expected values for the CA 15-3 Ag ELISA:

Healthy donors: ≤ **37 U/ml**

Each laboratory should establish its own expected values to its own patient population utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-CA153.pdf or

www.human-de.com/data/gb/vr/el-CA153.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

STOP **Warning**

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

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EL-CA153 INF 5208001 GB 02-2019-09M

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CA 19-9 ELISA

ELISA for the Quantitative Determination of Cancer Antigen 19-9 ~~(CA 19-9 Ag)~~ in Human Serum

Package Size

[REF]	52060	96 Tests	Complete Test Kit
[IVD]			

Intended Use

Cancer Antigen 19-9 was first discovered by a monoclonal antibody clone (1116NS 19-9) which selectively recognised human gastrointestinal carcinoma. CA 19-9 is a glycoprotein antigen determined as a modified Lewis (a) hapten of the human Lewis blood group system.

Recently reports indicate that serum CA 19-9 level is elevated in most patients with advanced pancreatic carcinoma, but may also be elevated in other gastrointestinal malignancies, such as colorectal, gastric and hepatic carcinomas. Combined with CEA elevated levels are also suggestive for gall bladder carcinoma.

CA 19-9 is neither carcinoma nor organ specific and therefore not indicated for screening, but it is a useful tool for differentiation between carcinoma of pancreas and bile ducts and other non-cancerous conditions. It is indicated for monitoring of therapy and patient progress.

Principle

The HUMAN CA 19-9 ~~Ag~~ ELISA intended for professional use is an ELISA for direct antigen detection including high affinity and specificity antibodies (enzyme labelled and biotinylated) with different and distinct epitope recognition. The assay makes use of the extremely high affinity of the system Biotin-Streptavidin. Streptavidin has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and Conjugate B (monoclonal, biotinylated antibody, mouse) are mixed to form an antibody-antigen complex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin.

After removing and washing out of unbound components Conjugate E (enzyme-labelled antibody, mouse) is added (step 2). The enzyme conjugate binds to the immobilised antibody-antigen complex forming the final sandwich complex. After incubation excess conjugate is washed out and TMB Substrate added (step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the CA 19-9 concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) breakable 8-well strips coated with streptavidin	
[CAL]	A - F 6x2 ml	Calibrators (imprinted, white cap) ready for use, human, yellowish CA 19-9 level: (A) 0 U/ml, (B) 10 U/ml, (C) 50 U/ml, (D) 100 U/ml, (E) 250 U/ml, (F) 500 U/ml	
[CON-B]	13 ml	Conjugate B (biotin) (white cap) Ready for use, coloured green monoclonal anti-CA 19-9 Ag antibody (mouse) biotinylated	
[CON-E]	13 ml	Conjugate E (enzyme) (white cap) Ready for use, coloured red monoclonal anti-CA 19-9 Ag (mouse) antibody HRP labelled	
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for about 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) Colourless 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	< 0.25 g/l 0.03 mol/l
[STOP]	8 ml	Stop Solution (red cap) Sulphuric acid, ready for use	0.5 mol/l
	2	Adhesive Strips	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found to be non-reactive. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with desiccant
- Must be at room temperature before opening
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution **[WASH]**

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic, hemolysed specimen or specimen containing sodium azide.

Specimens may be stored for 5 days at 2...8°C or up to 30 days at -20°C. Freeze and thaw twice only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of Microtiter Strips.

P5: Run duplicates or triplicates for positive resp. negative control. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the controls for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

P9: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents and add [WASH] to each well, aspirate off after 40-60 sec. soak time and repeat washing 2 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 40-60 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F in duplicate	25	--
Samples, controls in duplicate	--	25
[CON-B]	100	100
Mix and cover [MIC] with Adhesive Strips		
Incubate 60 min. at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[CON-E]	100	100
Do not shake [MIC] after [CON-E] addition		
[MIC] cover with Adhesive Strips		
Incubate 60 min. at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 3		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min. at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 620-630 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line) at 450 nm. The concentrations for patient samples are obtained by means of a calibration curve which is established from the calibrators supplied with the kit.

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] F ≥ 1.3.
- The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

The level of CA 19-9 cannot be used as absolute evidence for the presence or absence of malignant disease due to low clinical sensitivity and specificity of this tumor marker.

Determination of CA 19-9 is intended for monitoring of therapy and patient progress. The results should only be used in conjunction with other clinical investigations and procedures in the diagnosis and prognosis of disease.

The laboratory finding must always include a statement on the CA 19-9 method used due to potential variations in the result depending on the test procedure used. Therefore CA 19-9 values determined with different test procedures cannot be directly compared with one another. Change of assay procedure during monitoring of patients must be confirmed by parallel measurements with both procedures.

Expected values

CA 19-9 is elevated in malignant, but also in some benign conditions such as cirrhosis and other liver diseases, gall bladder disease, pancreatitis.

For interpretation of results it must it be taken into account that about 5% of the population are negative for the Lewis blood group antigens Lewis (a-b-). These patients do not express the CA 19-9.

Expected values for the CA 19-9 ~~Ag~~-ELISA:

Healthy donors: ≤ 40 U/ml

Each laboratory should establish its own expected values to its own patient population utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-CA199.pdf or

www.human-de.com/data/gb/vr/el-CA199.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

~~Note~~

~~The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!~~

~~(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)~~

Safety Notes

[STOP] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

1. Farini R. *et al.*, Eur. J. Cancer Clin. Oncol. 21, 429-432 (1985)
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6. Reiter W. *et al.*, anticancer Res. 20, 5195-5198 (2000)
7. Wild D., The Immunoassay Handbook, Stockton Press, 643 (2001)
8. Katsanos K.H. *et al.*, Eur. J. Int. Med. 13, 132-135 (2002)

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CEA

ELISA Test for the Quantitative Determination of Carcinoembryonic Antigen in Human Serum

Package Size

[REF]	52020	96 Tests	Complete Test Kit

[IVD]

Intended Use

Carcinoembryonic Antigen (CEA) is a glycoprotein with a molecular weight of 180 kDa synthesised during normal fetal development in gastrointestinal tract and pancreas and is secreted into the circulatory system. In adults CEA synthesis is not totally reduced. It can be detected in non-smokers with immunological methods.[1]

CEA is most widely used as marker of gastrointestinal cancer. Although CEA is primarily associated with colorectal cancer, other malignancies can cause elevated levels of CEA, which include breast, lung, stomach, ovary and other organs. Benign conditions that cause higher than normal levels include inflammation of lung, liver (cirrhosis) and gastrointestinal tract and benign cancer.[2,3] Heavy smokers, as a group, have a higher than normal baseline concentration of CEA.

Quantitative determination of CEA is used in monitoring patients with diagnosed malignancies, in whom elevated concentrations of CEA have been observed.[4]

Principle - Direct Antigen EIA -

The HUMAN CEA ELISA is intended for professional use. The ELISA for direct antigen detection uses the high affinity of Biotin for Streptavidin, which has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and antibody-enzyme conjugate (monoclonal anti-CEA antibodies peroxidase-labelled and monoclonal anti-CEA antibodies biotinylated) are mixed to form the sandwich complex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin. At the end of the incubation excess conjugate and unbound antigen are washed out. Substrate is added (Step 2), a blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the CEA concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with streptavidin	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, yellowish CEA level: 0 (A), 5 (B), 10 (C), 25 (D), 50 (E), and 250 (F) ng/ml	
[CON]	13 ml	Antibody-Enzyme Conjugate (white cap) ready for use, coloured yellow anti-CEA antibody (monoclonal, mouse) biotinylated, anti-CEA antibody (monoclonal, mouse) HRP-labelled	
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for approx. 1,000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	< 0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	25	--
Specimens, Controls; in duplicate	--	25
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known CEA concentrations.

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] F ≥ 1.3.
- The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

For years CEA has been the best known and most accepted tumour marker. It can be detected in a range of different tumours, but is of low clinical sensitivity and specificity (no tumour or organ specificity).

Patients with colorectal cancer may not exhibit elevated CEA values. Progression or regression of disease does not always result in elevated CEA levels. Smokers, but also elderly people, demonstrate a higher range of baseline values than non-smokers and younger persons.

CEA is an important marker for metastasis. High levels can be expected with bone, liver, pulmonary and multiple metastases.

Persistent high levels after treatment usually indicate residual malignant areas, or metastasis. A constant decrease is associated with favourable prognoses.

The determination of the CEA value alone is not sufficient to assess cancer. It should be used in conjunction with other clinical manifestations and diagnostic parameters.

Expected Values

	CEA level (1)
Non-smokers (99%)	< 5 ng/ml
Smokers	< 10 ng/ml

Each laboratory should establish its own Expected Values utilising in-strumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The CEA ELISA test has an analytical sensitivity of approximately 0.01 ng. This is equivalent to the CEA concentration of 0.45 ng/ml specimen.

Specimens with CEA concentrations higher than 250 ng/ml should be diluted 1+9 with normal serum (CEA < 5 ng/ml) and re-assayed. Multiply the result by 10.

The assay is standardised in accordance with WHO 1st I.R.P. for CEA (73/601): 1ng/ml = 0.011 IU/ml.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-cea.pdf or

www.human-de.com/data/gb/vr/el-cea.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

1. Wild D., The Immunoassay Handbook, Stockton Press, 444 (1994)
2. Zamcheck N., Adv. Intern. Med. 19, 413 (1974)
3. Rayncao G., Chu T., JAMA 220, 381 (1972)
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EL-CEA INF 5202001 GB 07-2015-21M

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Cortisol

ELISA Test for the Quantitative Determination of Cortisol in Human Serum or Plasma

Package Size

[REF]	55050	96 Tests	Complete Test Kit

[IVD]

Intended Use

Cortisol (hydrocortisone, compound F) a C_{21} steroid, with a molecular weight of 363.5 Dalton is the main glucocorticoid in humans, secreted by the adrenal cortex.

90% of the cortisol is bound to the plasmaprotein Transcortin and 7% to albumin. The remaining unbound part is the biological active form of cortisol to be determined in serum, plasma, urine or saliva.

Among the products of the adrenal cortex, only cortisol is involved in the regulation of ACTH secretion by a negative feedback mechanism.

Glucocorticoids act on the important physiological metabolisms of carbohydrates (e.g. gluconeogenesis, glycogen formation, increase in blood glucose), proteins (increase in catabolism, decrease in synthesis) and lipids. It is also involved in the inhibition of allergic and inflammatory reactions. Cortisol is essential for life, particularly when the human body is subject to stress such as surgery, major illness, or severe trauma.

The determination of cortisol is indicated in clinical diagnostics of abnormalities in glucocorticoid production[1], e.g. suspected Cushing's-Syndrome, Addison's disease or adrenal insufficiency.

Principle - Competitive EIA -

The HUMAN CORTISOL ELISA is intended for professional use. The ELISA is based on competitive interaction of cortisol and the hormone-enzyme conjugate for a limited number of monoclonal anti-cortisol antibodies (mouse). Thus, the amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of cortisol in the specimen.

After incubation of specimen and hormone-enzyme conjugate in the well, unbound conjugate is removed by washing. When substrate solution is added (step 2), a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is inversely proportional to the amount of cortisol in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) 8-well snap-off strips, coated with anti-cortisol antibodies (mouse)	
[CAL]	A - G 7x1.0ml	Calibrators (white cap) Ready to use, in human serum, slightly yellowish Cortisol - Level: 0 (A), 20 (B), 50 (C), 100 (D), 200 (E), 400 (F) and 800 (G) ng/ml	
[CON]	25 ml	Enzyme Antigen Conjugate (white cap) ready to use, coloured red	
		Cortisol – HRP – conjugate	pH 5.6 ± 0.1
		BSA	0.4 %
		TRIS/MES buffer	0.05 mol/l
		NaCl	0.1 mol/l
[WS]40x]	30 ml	Wash Solution (black cap)	
		Concentrate for ca. 1200 ml	pH 7.0 ± 0.1
		TWEEN 20	0.5 %
		TRIS buffered saline	3.0 mol/l
[SUB]	14 ml	Substrate Solution (yellow cap)	pH 3.5 – 4.0
		Ready to use	
		3,3', 5,5'-Tetramethylbenzidin (TMB)	0.26 g/l
		Hydrogen peroxide	0.015 %
		Sodium acetate buffer	0.05 mol/l
		DMSO	< 5 %
[STOP]	14 ml	Stop Solution (red cap)	
		Sulphuric acid	0.5 mol/l

Preservatives: Total concentration < 0.6%

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/630–690 nm filters, deionised water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 5 weeks.

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used for 60 days.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (21…26°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Dilute [WS]40x] to 1200 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 2 weeks, stored at 15...25°C.

Specimen

Serum or EDTA plasma

Do not use highly lipemic or hemolysed specimens and samples containing sodium azide.

Specimens may be stored for 3 days at 2...8°C, up to 30 days at 20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9: [MIC] - rock gently for 20-30 sec. after each pipetting step without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker (e.g. HumaReader).

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers fill and prime with [WASH] Sub-sequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3 After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...F2	G2...
[CAL] A-G; in duplicate	20	--
Specimens, Controls; in duplicate	--	20
[CON]	200	200
Mix		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1/W2 + W3)		
[WASH]	400	400
Step 2		
[SUB]	100	100
Incubate 15 min at 20...25°C (see P8)		
[STOP]	100	100
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after termination of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known cortisol concentrations.

Validation of the Test

The test results are valid provided the following criteria are met:

- The mean absorbance (OD) of [CAL] A ≥ 1.0.
- The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points results in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Controls should be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

Cortisol shows a circadian rhythm with high levels in the morning and lower levels at night. Therefore, only cortisol values of samples withdrawn at a fixed hour (usually 8:00 a.m.) give useful information (see Expected Values).

Increased cortisol levels (hypercortisolism) above the Expected Values are suspected for Cushing's Syndrome, which is indicated for patients with irregular transcortin concentration (e.g. adiposity, pregnancy, hypothyroidism). But values within the Expected Values may not exclude Cushing's Syndrome[2].

Hypocortisolism, cortisol levels below the basal values for repeated determinations, are suspected for primary and secondary adrenal insufficiency, which should be confirmed by testing of ACTH.[3]

The cortisol value alone is not sufficient for differentiation and diagnosis of pathological conditions. It should be used in conjunction with other clinical manifestations and other diagnostic (hormone) parameters.

Expected Values

8:00 a.m.	Cortisol level
Children[4]	
≤ 5 days	6 – 200 ng/ml
2-12 months, 2-15 years	24 – 230 ng/ml
16-18 years	24 – 290 ng/ml
Adults[5]	50 – 250 ng /ml

Conversion factor: 1 ng/ml = 2.76 nmol/l

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The CORTISOL ELISA test has an analytic sensitivity of 2.5 ng/ml specimen.

Specimens with cortisol concentrations above 800 ng/ml should be diluted (1+9) with [CAL] A (0 ng/ml) and re-assayed. Multiply the result by 10.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-cort.pdf or

www.human-de.com/data/gb/vr/el-cort.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

[CON] may contain black particles. This does not influence the assay performance.

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 5 weeks limit after first use is set for safety reasons.

Safety Notes

~~- Hazard statements~~

[STOP] [WS]40x] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[WS]40x] Warning

H335 May cause respiratory irritation.

~~[SUB] Danger~~

~~- Hazard statements~~

~~H360D May damage the unborn child.~~

~~- Precautionary statements~~

[CAL] [CON] [WS[40x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with

water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.~~

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

References

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DHEA-S

ELISA Test for the Quantitative Determination of Dehydroepiandrosterone Sulfate in Human Serum or Plasma

Package Size

[REF]	55060	96 Tests	Complete Test Kit

[IVD]

Intended Use

Dehydroepiandrosterone (DHEA) is the major C_{19} adrenal steroid secreted by the adrenal cortex. Most of the DHEA in serum is present in the sulphate form DHEA-S and bound to albumin.

DHEA-S is significantly involved in the process of androgenesis. The weak androgen DHEA-S, in terms of biological activity, is converted into the much stronger Testosterone and Estrogens in peripheral tissues.

DHEA-S is an important indicator for the androgen production of the adrenal cortex and plays an important role for the fetoplacental unit. In case of suspected retardation of fetal growth functional stress testing with DHEA-S is indicated.

Determination of DHEA-S in conjunction with Cortisol provides the opportunity for differential diagnosis of the adrenocortical function, because of production of both steroids by the adrenal cortex, but in different zones. Furthermore DHEA-S concentration in serum is directly correlated to urine 17-Ketosteroids, because of its higher concentration DHEA-S is the more sensitive indicator for routine determination of adrenal androgens.

Evaluation of serum DHEA-S is indicated for diagnosis of adrenal hyperplasia (Cushings Syndrome, Adrenogenital Syndrome), hirsutism and virilising adrenal tumours[1].

Principle - Competitive EIA -

The HUMAN DHEA-S ELISA is intended for professional use. The ELISA is based on competitive interaction of DHEA-S and the hormone-enzyme conjugate for a limited number of immobilised anti-DHEA-S antibodies (rabbit). Thus, the amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of DHEA-S in the specimen.

After incubation of specimen and hormone-enzyme conjugate in the well, unbound conjugate is removed by washing. When substrate solution is added (step 2), a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is inversely proportional to the amount of DHEA-S in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) 8-well snap-off strips, coated with anti-DHEA-S anti-bodies (rabbit)	
[CAL]	A - G 7x1.0ml	Calibrators (white cap) Ready to use, in human serum, yellowish DHEA-S- Level: 0 (A), 0.1 (B), 0.5 (C), 1.0 (D), 2.5 (E), 5.0 (F) and 10 (G) µg/ml	
[CON]	25 ml	Enzyme Antigen Conjugate (white cap) ready to use, coloured red	
		DHEAS – HRP – conjugate	pH 6.9 ± 0.2
		BSA	0.5 %
		TRIS/MOPS buffer	0.05 mol/l
		NaCl	0.1 mol/l
[WS]40x]	30 ml	Wash Solution (black cap) Concentrate for ca. 1200 ml	pH 7.0 ± 0.1
		TWEEN 20	0.5 %
		TRIS buffered saline	3.0 mol/l
[SUB]	14 ml	Substrate Solution (yellow cap) Ready to use	pH 3.5 – 4.0
		3,3', 5,5'-Tetramethylbenzidin (TMB)	0.26 g/l
		Hydrogen peroxide	0.015 %
		Sodium acetate buffer	0.05 mol/l
		DMSO	< 5 %
[STOP]	14ml	Stop Solution (red cap) Sulphuric acid	0.5 mol/l

Preservatives: Total concentration < 0.7%

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/630–690 nm filters, deionised water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used for 60 days.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution **[WASH]**

- Dilute [WS]40x] to 1200 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 2 weeks, stored at 15...25°C.

Specimen

Serum or EDTA plasma

Do not use highly lipemic or hemolysed specimens and samples containing sodium azide.

Specimens may be stored for 3 days at 2...8°C, up to 30 days at - 20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1 : Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2 : Do not use reagents that could be contaminated or look or smell different than usual.

P3 : Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4 : [MIC] - select the required number and place firmly in the holder.

P5 : Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6 : Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7 : Avoid/remove air bubbles prior to incubations and reading absorbance.

P8 : [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9 : [MIC] - rock gently for 20-30 sec. after each pipetting step without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker.

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1 : Aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2 : In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3 : After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...F2 [CAL]	G2... Sample
[CAL] A-G; in duplicate	25	--
Specimens, controls; in duplicate	--	25
[CON]	200	200
Mix		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1/W2 + W3)		
[WASH]	400	400
Step 2		
[SUB]	100	100
Incubate 15 min at 20...25°C (see P8)		
[STOP]	100	100
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after termination of reaction, using a reference wavelength of 630 - 690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN´s HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known DHEA-S concentrations.

Validation of the Test

The test results are valid provided the following criteria are met:

- The mean absorbance (OD) of [CAL] A ≥ 1.0.
- The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points results in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

DHEA-S shows no diurnal or day to day variations because of its slow metabolic clearance.

DHEA-S values are high at birth, fall rapidly during childhood, grow gradually and continuously during prepuberty and reach adult levels shortly after puberty. Increase in age (after third decade) results in decline of DHEA-S for both sexes. The values from men are higher than values from women.[2] Pregnant women show a reduction of initial values by about 50% due to placental clearance.

Increased values of DHEA-S are an indication for adrenal hyperplasia and adrenal tumours, but also for adrenal hirsutism. Androgenism of women caused by polycystic ovaries may be indicated by values of 3-7 µg/ml. Cancer of female patients is suspected at > 7 µg/ml.

Determination of DHEA-S and Testosterone provides the opportunity of differentiation of androgen producing tumours from other diseases by testing of sera from ovary and adrenal cortex[3].

Determination of the DHEA-S value alone is not sufficient for diagnosis of pathological conditions. It should be used in conjunction with other clinical manifestations and diagnostic (hormone) parameters.

Expected Values

	DHEA-S level
Normal women[4]	
Premenopause	0.8 – 3.9 µg/ml
Term Pregnancy	0.2 – 1.2 µg/ml
Postmenopause	0.1 – 0.6 µg/ml
Normal men[4]	1.0 – 4.2 µg/ml
Newborns (both sexes)[4]	1.7 – 3.6 µg/ml
Children[5]	
1-5 years	< 0.3 µg/ml
6-11 years	0.1 – 1.5 µg/ml
12-17 years	0.3 – 5.5 µg/ml

Conversion factor: 1 µg/ml = 2.6 µmol/l

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The DHEA-S ELISA test has an analytic sensitivity of 0.01 – 0.03 µg/ml specimen.

Specimens with DHEA-S concentrations above 10 µg/ml should be diluted (1+9) with [CAL] A (0 µg/ml) and re-assayed. Multiply the result by 10.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-dhea.pdf or
www.human-de.com/data/gb/vr/el-dhea.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

Safety Notes

~~- Hazard statements~~

[STOP] [WS]40x] Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[WS]40x] Warning

H335 May cause respiratory irritation.

~~[SUB] Danger~~

~~- Hazard statements~~

~~H360D May damage the unborn child.~~

~~- Precautionary statements~~

[CAL] [CON] [WS][40x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with

water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.~~

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

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Estradiol

ELISA Test for the Quantitative Determination of Estradiol in Human Serum or Plasma

Package Size

[REF]	55030	96 Tests	Complete Test Kit
[IVD]			

Intended Use

Estradiol (1,3,5(10)-estratriene-3,17β–diol; 17β-estradiol; E2) is a C_{18} steroid hormone with a molecular weight of 272.4 Dalton containing a phenolic A ring. It is the most important natural estrogen to be detected in women and also in men.[1, 2]

Estradiol is produced by follicles of the female ovary after stimulation by the Follicle Stimulating Hormone (FSH) and is secreted into the circulation. About 98% of the circulating hormone is bound, the remaining portion, the free estradiol, is assumed to be the active steroid.[3] It is involved in gonadotropin secretion and stimulates the growth of sex organs and the development of secondary sexual characteristics.

In normal menstrual cycles of non-pregnant women estradiol is secreted cyclic with a biphasic characteristic. The highest concentration will be detected prior to ovulation.[4] Pregnant women show an increased level of estradiol, much higher than in normal menstrual cycles.[5]

In men estradiol is produced by the adrenal gland and testes. Its role is less well defined, but it seems to be involved in the regulation of gonadotropins.

Measurement of estradiol is indicated for therapeutic monitoring in case of infertility (e.g. hormone treatment or “in vitro” fertilisation). It’s also helpful for the assessment of ovarian function, but seldom used for diagnostic of tumours.

Principle - Competitive EIA -

The HUMAN ESTRADIOL ELISA is intended for professional use. The ELISA is based on competitive interaction of estradiol and the hormone-enzyme conjugate for a limited number of immobilised anti-estradiol antibodies (rabbit). Thus, the amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of estradiol in the specimen.

After incubation of specimen and hormone-enzyme conjugate in the well, unbound conjugate is removed by washing. When substrate solution is added (step 2), a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is inversely proportional to the amount of estradiol in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) 8-well snap-off strips, coated with anti-estradiol anti-bodies (rabbit)	
[CAL]	A - G 7x1.0ml	Calibrators (white cap) Ready to use, in human serum, yellowish Estradiol - Level: 0 (A), 25 (B), 100 (C), 250 (D), 500 (E), 1000 (F) and 2000 (G) pg/ml	
[CON]	14 ml	Enzyme Antigen Conjugate (white cap) ready to use, coloured red	
		Estradiol – HRP – conjugate	pH 6.9 ± 0.2
		BSA	0.5 %
		TRIS/MOPS buffer	0.05 mol/l
		NaCl	0.1 mol/l
[WS]40x]	30 ml	Wash Solution (black cap)	
		Concentrate for ca. 1200 ml	pH 7.0 ± 0.1
		TWEEN 20	0.5 %
		TRIS buffered saline	3.0 mol/l
[SUB]	14 ml	Substrate Solution (yellow cap) Ready to use	pH 3.5 – 4.0
		3,3', 5,5'-Tetramethylbenzidine (TMB)	0.26 g/l
		Hydrogen peroxide	0.015 %
		Sodium Acetate buffer	0.05 mol/l
		DMSO	< 5 %
[STOP]	14ml	Stop Solution (red cap)	
		Sulphuric acid	0.5 mol/l

Preservatives: Total concentration < 0.7%

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/~~630-690~~620 – 630 nm filters, destilled~~deionised~~ water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within ~~60 days~~8 weeks.

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used for 60 days.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Dilute [WS]40x] to 1200 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 2 weeks, stored at 15...25°C.

Specimen

Serum or ~~EDTA~~ plasma (EDTA, citrate, heparin)

Do not use highly haemolytic, icteric or lipemic specimens and samples containing sodium azide.

Do not use if patients are treated with the drug fulvestrant (Faslodex®), as cross reactions may lead to falsely elevated test results.

Specimens may be stored for ~~3~~7 days at 2...8°C, up to ~~30 days~~12 months at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9: [MIC] - rock gently for 20-30 sec. after each pipetting step without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker.

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...F2	G2...
[CAL] A-G; in duplicate	25	--
Specimens, controls; in duplicate	--	25
[CON]	100	100
Mix		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1/W2 + W3)		
[WASH]	400	400
Step 2		
[SUB]	100	100
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 10 min. after termination of reaction, using a reference wavelength of ~~630 690~~620 – 630 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN' HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known estradiol concentrations.

Validation of the Test

The test results are valid provided the following criteria are met:

- The mean absorbance (OD) of [CAL] A ≥ 1.0.
- The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points results in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

Determination of serum estradiol is indicated for a variety of menstrual dysfunctions such as precocious or delayed puberty of girls[11] and primary and secondary amenorrhea and menopause[7]. Reduced estradiol levels may be detected for insufficiency of ovaries (< 10 pg/ml) or corpus luteum, but also for anovulatory cycles.

Serum estradiol measurements are important for monitoring of ovulation induced e.g. with LH-releasing hormone (LH-RH).[8] For “in vitro” fertilisation (IVF) the concentration of serum estradiol is usually monitored daily for optimal timing of human chorionic gonadotropin administration (hCG) and oocyte collection.[9]

Elevated estradiol level has been detected in patients with feminising syndromes, gynaecomastia[10] and testicular tumors.[11]

Determination of the estradiol value alone is not sufficient for diagnosis of pathological conditions. It should be used in conjunction with other clinical manifestations and diagnostic (hormone) parameters.

Expected Values

	Estradiol level
Normal women:	
Follicular phase	28 – 178 pg/ml
Ovulatory peak	51 – 549 pg/ml
Luteal phase	34 – 251 pg/ml
Menopause	18 – 64 pg/ml
Normal men:	30 – 68 pg/ml

Conversion factor: 1 pg/ml = 3.67 pmol/l

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The ESTRADIOL ELISA test has an analytic sensitivity of 13 pg/ml specimen.

Specimens with estradiol concentrations above 2000 pg/ml should be diluted (1+9) with [CAL] A (0 pg/ml) and re-assayed. Multiply the result by 10.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-estra.pdf or

www.human-de.com/data/gb/vr/el-estra.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

Safety Notes

[STOP] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[WS]40x] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

· Precautionary statements

[CAL] [CON] [WS]40x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.

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hCG

ELISA Test for the Quantitative Determination of Human Chorionic Gonadotropin (hCG) in Human Serum

Package Size

[REF]	53040	96 Tests	Complete Test Kit

[IVD]

Intended Use

Human chorionic gonadotropin (hCG) is a glycoprotein hormone, secreted by trophoblastic cells of the placental tissue. The major physiological role of the hormone is to support the corpus luteum during pregnancy stages. HCG is the most important marker for pregnancy. While the hCG level in non-pregnant females and healthy males typically is below 1 - 2 IU/l, hCG rises early after conception and may reach levels of 10 - 30 IU/l within the first week of pregnancy. During normal pregnancy the hCG values double each 1.3 - 2 days, reaching a peak level between week 8 - 12. After this peak the hCG level slowly declines to a plateau which is maintained throughout pregnancy. Following delivery the hCG level rapidly declines to normal.

Principle - Direct Antigen EIA -

The HUMAN hCG ELISA is intended for professional use. The ELISA for direct antigen detection uses the high affinity of Biotin for Streptavidin, which has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and antibody-enzyme conjugate (monoclonal anti-hCG antibodies peroxidase-labelled or biotinylated are mixed to form a specific immunocomplex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin. At the end of the incubation excess conjugate and unbound antigen are washed out. TMB/Substrate is added (Step 2), a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the hCG concentration in the specimen.

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known hCG concentrations.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with streptavidin	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, yellowish hCG level: 0 (A), 5 (B), 25 (C), 50 (D), 100 (E), and 250 (F) IU/l	
[CON]	13 ml	Antibody-Enzyme Conjugate (white cap) ready for use, coloured blue anti-hCG (monoclonal, mouse) HRP-labelled and anti-hCG (monoclonal, mouse), biotinylated	1.0 µg/ml
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for approx. 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium Acetate buffer	< 0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric Acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipaemic or haemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	25	--
Specimens, Controls; in duplicate	--	25
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] F ≥ 1.3.
- The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

As hCG may also be secreted by a variety of trophoblastic and non-trophoblastic neoplasms, such cases should be ruled out before diagnosing pregnancy.

Patients under hormone therapy may exhibit false positive results due to the uptake of hCG or stimulation of hCG secreting cells. Spontaneous microabortions and induced therapeutic abortions may lead to a rapid decrease and consequently low hCG levels, though in certain cases hCG may persist for some weeks.

Ectopic pregnancies usually exhibit lower hCG values compared with normal pregnancies. However, due to wide physiological variations a single determination will not be sufficient to diagnose ectopic from normal pregnancy. In such cases it has been recommended to determine the doubling rate of hCG values which should be around 1.3 - 2 days in normal pregnancies. Delayed doubling rates may be an indication for ectopic pregnancy. The final diagnosis should not be based only on the hCG determinations. Other diagnostic procedures have to be included.

Expected Values

HCG levels during normal pregnancy

Time after conception	hCG [IU/l]
1st week	10 - 30
2nd week	30 - 100
3rd week	100 - 1000
4th week	1000 - 10000
2nd - 3rd month	30000 - >100000
2nd trimester	10000 - 30000
3rd trimester	5000 - 15000

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The hCG ELISA test has a analytic sensitivity of about 0.8 IU/l hCG.

Specimens with hCG concentrations higher than 250 IU/l may be diluted with normal male serum and re-assayed. To obtain the sample's concentration multiply by the dilution factor.

The assay is standardised in accordance with WHO 3rd IS for hCG (75/537).

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-hcg.pdf or

www.human-de.com/data/gb/vr/el-hcg.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

1. Kosasa T. S., Journal of Reproductive Medicine 26, 201-6 (1981)
2. Danzer H. *et al.*, Fertility and Sterility 34, 336-40 (1980)
3. Braunstein G. D. *et al.*, American Journal of Obstetrics and Gynecology 126, 678-81 (1976)
4. Goldstein D. P., Kosasa T. S., Clinical Application, Gynecology 6, 145-84 (1975)
5. Batzer F., Fertility and Sterility 34, 1-12 (1980)
6. Braunstein G. D. *et al.*, American Journal of Obstetrics and Gynecology 131, 25-32 (1978)

EL-HCG INF 5304001 GB 07-2015-25M

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FSH

ELISA Test for the Quantitative Determination of Follicle Stimulating Hormone (FSH) in Human Serum

Package Size

[REF]	53020	96 Tests	Complete Test Kit

[IVD]

Intended Use

Follicle stimulating hormone (FSH) is a glycoprotein hormone of approx. 35.5 kD, secreted by the pituitary gland. Physiologically this hormone acts on the testes and ovary, respectively, where it supports spermatogenesis (in men) and steroidogenesis (in women). All menstrual cycles have a characteristic pattern of FSH secretion. While FSH concentration decreases with progressing follicular phase, peak values are observed near the ovulation time, i.e. about midcycle. The clinical and diagnostic usefulness of FSH measurement for ascertaining the homeostasis of fertility regulation via the hypothalamic-pituitary-gonadal axis has been well established.

Principle - Direct Antigen EIA -

The HUMAN FSH ELISA is intended for professional use. The ELISA for direct antigen detection uses the high affinity of Biotin for Streptavidin, which has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and enzyme conjugate (peroxidase-labelled anti-FSH and biotinylated monoclonal anti-FSH) are mixed to form a specific immunocomplex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin. At the end of the incubation excess enzyme conjugate and monoclonal antibodies are washed out. TMB/Substrate is added (step 2) and the resulting colour, which turns into yellow after stopping the reaction with the stop solution, is measured photometrically. The intensity of colour is directly proportional to the FSH concentration in the sample.

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). The concentration is evaluated by means of a calibration curve which is established from the calibrators supplied with the kit

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with streptavidin
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, yellowish FSH level: 0 (A), 5 (B), 10 (C), 25 (D), 50 (E), and 100 (F) IU/l
[CON]	13 ml	Enzyme Conjugate (white cap) ready for use, coloured greenish-blue anti-FSH (monoclonal, mouse), HRP-labelled, anti-FSH (monoclonal, mouse), biotinylated 1.0 µg/ml
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for approx. 1000 ml Tris buffered saline 250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) < 0.25 g/l Sodium acetate buffer 0.03 mol/l Hydrogen peroxide
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric acid 0.5 mol/l
	1	Adhesive strip

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	50	--
Specimens, Controls; in duplicate	--	50
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] F ≥ 1.3.
- The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

FSH concentration depends upon diverse factors other than pituitary homeostasis. Thus, the determination of FSH alone is not sufficient to assess the clinical status.

FSH is suppressed by estrogens; still in women under oral contraceptives the FSH levels may be normal.

Excessive dieting and weight loss may lead to low FSH concentrations.

Expected Values

FSH levels during normal menstrual cycle

Cycle phase	FSH [IU/l]
Follicular phase	3.0 - 12.0
Midcycle	8.0 - 22.0
Luteal phase	2.0 - 12.0
Postmenopausal	35.0 - 151

In men, FSH values of 1.0 - 14.0 IU/l have been reported.

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The FSH ELISA test has an analytic sensitivity of about 0.8 IU/l FSH. Specimens with concentrations exceeding 100 IU/l should be diluted by mixing 25 µl of the specimen with 25 µl of [CAL] A (0.0 IU/l FSH) in the respective sample wells. Multiply the result by 2.

The assay is standardised in accordance with WHO 2nd IRP (78/549).

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-fsh.pdf or

www.human-de.com/data/gb/vr/el-fsh.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

1. Odell W.D. *et al.*, J. Clin. Investigation 47, 2551 1981)
2. Saxema B.B. *et al.*, J. Clin. Endocrinology and Metabolism 28, 591 (1968)

EL-FSH INF 5302001 GB 07-2015-23M

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LH

ELISA Test for the Quantitative Determination of Luteinizing Hormone (LH) in Human Serum

Package Size

[REF]	53010	96 Tests	Complete Test Kit

[IVD]

Intended Use

Luteinizing hormone (LH) is a glycoprotein hormone of approx. 30 kD, which is synthesised in the hypophysis. Physiologically this hormone acts on the gonads and is essential for their development and function. During the first phase of the menstrual cycle LH stimulates the progress of the follicle to the Graaf's follicle. Ovulation is induced by peak levels of LH. The clinical and diagnostic usefulness of LH measurement for ascertaining the homeostasis of fertility regulation via the hypothalamic-pituitary-gonadal axis has been well established. LH determination is indispensable for monitoring subjects who undergo in vitro fertilisation.

Principle - Direct Antigen EIA -

The HUMAN LH ELISA is intended for professional use. The ELISA for direct antigen detection uses the high affinity of Biotin for Streptavidin, which has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and enzyme conjugate (peroxidase-labeled x-LH and biotinylated monoclonal anti-LH) are mixed to form a specific immunocomplex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin. At the end of the incubation excess enzyme conjugate and monoclonal antibodies are washed out. TMB/Substrate is added (step 2) and the resulting colour, which turns into yellow after stopping the reaction with the stop solution, is measured photometrically. The intensity of colour is directly proportional to the LH concentration in the sample.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with streptavidin	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, yellowish LH level: 0 (A), 5 (B), 25 (C), 50 (D), 100 (E), and 200 (F) IU/l	
[CON]	13 ml	Enzyme Conjugate (white cap) ready for use, coloured orange x-LH (monoclonal, mouse), HRP-labelled, anti-LH (monoclonal, mouse), biotinylated	 1.0 µg/ml
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for approx. 1000 ml Tris buffered saline	 250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	 < 0.25 g/l 0.03 mol/l
[STOP	7.5 ml	Stop solution (red cap) Sulphuric acid	 0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or haemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	50	--
Specimens, Controls; in duplicate	--	50
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). The concentration is evaluated by means of a calibration curve which is established from the calibrators supplied with the kit.

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] F ≥ 1.3.
- The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

LH depends upon diverse factors other than pituitary homeostasis. Thus, the determination of LH alone is not sufficient to assess the clinical status.

LH is suppressed by estrogens; still in women under oral contraception the LH levels may be normal.

Excessive dieting and weight loss may lead to low LH concentrations.

Expected Values for LH Levels throughout Normal Menstrual Cycle

Cycle phase	LH [IU/l]
Follicular phase	0.8 - 10.5
Midcycle	18.4 - 61.2
Luteal phase	0.8 - 10.5
Postmenopausal	8.2 - 40.8

In men, LH values of 0.7 - 7.4 IU/l have been reported.

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The LH ELISA test has an analytic sensitivity of about 0.8 IU/l LH.

Specimens with LH concentrations higher than 200 IU/l may be diluted with normal male serum and re-assayed. To obtain the sample's concentration multiply by the dilution factor.

The assay is standardised in accordance with WHO IRP for LH (68/40).

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-lh.pdf or

www.human-de.com/data/gb/vr/el-lh.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

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2. Danzer H. *et al.*, Fertility and Sterility 34, 336-40 (1980)
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EL-LH INF 5301001 GB 07-2015-23M

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Total IgE

ELISA for the Quantitative Determination of Total IgE in Human Serum

Package Size

[REF]	51015	96 Tests	Complete Test Kit
[IVD]			

Intended Use

Allergic reactions, for example asthma, dermatitis and pollen allergy are usually diagnosed on the basis of medical history and clinical symptoms.

The measurement of the immunoglobulin E (IgE) is very important in confirming the clinical suspicion.

High IgE concentrations can direct to an IgE hypersensitivity which can cause different allergic reactions. Furthermore, some parasitic infections can also increase the IgE level.

The included IgE Standards are compared with International Reference Units (3rd international Standard 11/234). The results can be reported in IU/ml.

Principle - Direct Antigen EIA -

The HUMAN TOTAL IgE ELISA intended for professional use is based on the direct antigen ELISA technique. A monoclonal anti-human IgE antibody has been coated on the surface of microtiter wells.

In the first incubation step, specimens, calibrators and anti-IgE conjugate are pipetted into the wells of the microtiter plate. A sandwich complex between the antibodies of the serum, the immobilised antibody and the anti-IgE conjugate takes place. At the end of the incubation unbound components are washed out.

TMB/Substrate is added, a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the total IgE concentration in the specimen.

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known total IgE concentrations.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with monoclonal Anti-IgE	
[CAL]	A – F 1 ml 5x 0.4 ml	IgE Calibrators, ready to use, in human serum IgE Level: 0 (A) 5 (B), 25 (C), 100 (D), 250 (E) and 1000 (F) lU/ml	
[CON]	22 ml	Anti-IgE-Conjugate ready to use, coloured red anti-human-IgE (goat) peroxidase-conjugated	
[WS]10x]	2x60 ml	Wash Solution Concentrate for ca. 1200 ml PBS Puffer with Tween 20	pH 6.5 – 7.0
[SUB]	13 ml	Substrate Reagent (brown bottle) 3,3', 5,5'-Tetramethylbenzidine (TMB)	
[STOP]	13ml	Stop Solution Sulphuric acid, ready to use	0.5 mol/l
	2	Adhesive strips	

Preservatives: Total concentration < 0.1%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 3 months.

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant and store in this way at 2...8°C.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Dilute [WS]10x] 1+9 with fresh deionised water, e.g. 30 ml [WS]10x] + 270 ml deionised water = 300 ml
- Stability: 4 weeks at 2...8°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at - 20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL] and the specimen. Pipette [CAL] and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] – incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents into 5% sodium hypochlorite solution, add [WASH], aspirate off after 30 sec. soak time and repeat washing 2 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.			
Step 1	*Well [µl]*		
	A1 Blank	B1...E2 [CAL]	F2... Specimen
[CAL] A-F; in duplicate	--	10	--
Undiluted specimens in duplicate	--	--	10
[CON]	--	200	200
Cover [MIC] with Adhesive Strip			
Incubate 30 min at 17...25°C			
Wash 3 times as described (see W1 - W3)			
[WASH]	300	300	300
Step 2			
[SUB]	100	100	100
Incubate 15 min at 17...25°C (see P8)			
[STOP]	100	100	100
Mix carefully			
Zero the ELISA microtiter plate reader (HumaReader) using the substrate blank in well A1.			
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 630 - 690 nm (if available).			

Calculation of the Results

The mean absorbance values are calculated after subtraction of the substrate blank value (well A1)

The test run may be considered valid provided that the following criteria are met:

Standard [IU/ml]	Accepted range [OD]
0	≤ 0.100
5	≥ 0.015
25	≥ 0.050
100	≥ 0.150
250	≥ 0.400
1000	≥ 1.000

The differences between the duplicates of [CAL] should not exceed 10%.

A dose response curve is used to interpolate the concentration of total IgE in unknown specimens.

1. [CAL] - Plot the absorbance for each duplicate versus the corresponding IgE concentration in IU/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
2. Draw the best fit curve through the plotted points.
3. To determine the concentration of IgE for an unknown sample, locate the average absorbance of the duplicate on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph.

Interpretation of the Total IgE Results for Adults

IU/ml	Interpretation
< 25	no indication of an allergic reaction
25 – 100	allergy possible - confirmation of diagnosis recommended (specific IgE)
100	allergy very probable

Total IgE Reference Ranges of Healthy Children

	Age		IU/ml
Baby	0 – 10	Days	< 0.5
Infant	0.5 – 2	Years	0.6 – 9.6
	2 – 5	Years	1.6 – 33
	5 – 8	Years	0.8 – 52
	8 – 12	Years	0.7 – 86
	12 – 16	Years	1.3 – 70

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-ige.pdf or

www.human-de.com/data/gb/vr/el-ige pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

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1. Liappis N., D. Berdel, Referenzbereiche für die IgE-Konzentration im Serum von Kindern ;Extracta Diagnostica 30 (1989)
2. Clinical Laboratory Diagnostics, Total IgE, German Edition, 953-956 (1992)
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Progesterone

ELISA Test for the Quantitative Determination of Progesterone in Human Serum or Plasma

Package Size

[REF]	55020	96 Tests	Complete Test Kit

[IVD]

Intended Use

Progesterone (pregn-4-ene-3, 20-dione) is a C_{21} steroid hormone with a molecular weight of 314.5 Dalton. It is the most important hormone (progestogen) produced in non-pregnant women by the corpus luteum, in pregnant women by the placenta. Minor sources are in men the testes and in both sexes the adrenal cortex.

During the menstrual cycle progesterone regulates, together with estrogens (estradiol), accessory organs. Furthermore it is involved in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy.

Progesterone circulates in blood bound to binding globulins (CBG, SHBG) and albumin. About 90-98% of the circulating hormone is bound, the remaining portion, the free progesterone, is assumed to be the active steroid.

The progesterone level in blood varies widely according to the phases of the menstrual cycle and parallels the activity of ovarian follicle and corpus luteum. Therefore determination of progesterone is clinically important for confirmation of ovulation and normal function of the corpus luteum in non-pregnant women.[1]

Abnormal progesterone secretion[2,3,4,5] may be implicated in pre-menstrual tension, luteal insufficiency, dysmenorrhoea and irregular shedding of endometrium.

Principle - Competitive EIA -

The HUMAN PROGESTERONE ELISA is intended for professional use. The ELISA is based on competitive interaction of progesterone and the hormone-enzyme conjugate for a limited number of immobilised anti-progesterone antibodies (rabbit). Thus the amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of progesterone in the specimen.

After incubation of specimen and hormone-enzyme conjugate in the well, unbound conjugate is removed by washing. When substrate solution is added (step 2), a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is inversely proportional to the amount of progesterone in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) 8-well snap-off strips, coated with anti-progesterone anti-bodies (rabbit)	
[CAL]	A - G 7x1.0ml	Calibrators (white cap) Ready to use, in human serum, yellowish Progesterone - Level: 0 (A), 0.3 (B), 1.25 (C), 2.5 (D), 5.0 (E), 15.0 (F) and 40.0 (G) ng/ml	
[CON]	25 ml	Enzyme Antigen Conjugate (white cap) ready to use, coloured red	
		Progesterone – HRP – conjugate	pH 6.9 ± 0.2
		BSA	0.5 %
		TRIS/MOPS buffer	0.05 mol/l
		NaCl	0.1 mol/l
[WS]40x]	30 ml	Wash Solution (black cap) Concentrate for ca. 1200 ml	pH 7.0 ± 0.1
		TWEEN 20	0.5 %
		TRIS buffered saline	3.0 mol/l
[SUB]	25 ml	Substrate Solution (yellow cap) Ready to use	pH 3.5 – 4.0
		3.3', 5.5'-Tetramethylbenzidine (TMB)	0.26 g/l
		Hydrogen peroxide	0.015 %
		Sodium acetate buffer	0.05 mol/l
		DMSO	< 5 %
[STOP]	14ml	Stop Solution (red cap) Sulphuric acid	0.5 mol/l

Preservatives: Total concentration < 0.7%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used for 60 days (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Dilute [WS]40x] to 1200 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 2 weeks, stored at 15...25°C.

Specimen

Serum or EDTA plasma

Do not use highly lipemic or hemolysed specimens and samples containing sodium azide.

Specimens may be stored for 3 days at 2...8°C, up to 30 days at - 20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9: [MIC] - rock gently for 20-30 sec. after each pipetting step without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...F2	G2...
[CAL] A-G; in duplicate	25	--
Specimens, controls; in duplicate	--	25
[CON]	200	200
Mix		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1/W2 + W3)		
[WASH]	400	400
Step 2		
[SUB]	200	200
Incubate 15 min at 20...25°C (see P8)		
[STOP]	100	100
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after termination of reaction, using a reference wavelength of 630 - 690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of un-known specimen is interpolated from a dose response curve generated by utilising serum calibrators of known progesterone concentrations.

Validation of the Test

The test results are valid provided the following criteria are met:

- The mean absorbance (OD) of [CAL] A ≥ 1.0.
- The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges

Interpretation of Results

Progesterone concentration may vary in a single person from day to day or even from hour to hour. Therefore serial determinations are recommended for a proper interpretation of the results in cases for gynaecological disorders or abnormal pregnancies.

Progesterone has a thermogenetic effect and induces an increase in basal temperature. The interpretation of progesterone values will therefore be easier in the context of a basal temperature curve.

The ranges of progesterone vary from less than 1 ng/ml in the follicular phase to around 10-20 ng/ml in the luteal phase (see Expected Values). Maximal levels are reached 6-8 days after ovulation and elevated for 4-6 days. They fall to former low levels 1-2 days before onset of menstruation.

During pregnancy the concentration of progesterone produced by the placenta increases constantly by reaching a peak value of 200 ng/ml.

Determination of the progesterone value alone is not sufficient for diagnosis of pathological conditions. It should be used in conjunction with other clinical manifestations and diagnostic (hormone) parameters.

Expected Values

	Progesterone level
Normal women:	
Follicular phase	0.2 – 1.4 ng/ml
Luteal phase	4 – 25 ng/ml
Menopause	0.1 – 1 ng/ml
Normal men:	0.1 – 1 ng/ml

Conversion factor: 1 ng/ml = 3.18 nmol/l

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristic

The PROGESTERONE ELISA test has an analytic sensitivity of 0.03 - 0.07 ng/ml specimen.

Specimens with progesterone concentrations above 40 ng/ml should be diluted (1+9) with [CAL] A (0 ng/ml) and re-assayed. Multiply the result by 10.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-prog.pdf or

www.human-de.com/data/gb/vr/el-prog.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

[WS]40x] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

· Precautionary statements

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

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PRL

ELISA Test for the Quantitative Determination of Prolactin (PRL)

Package Size

[REF]	53030	96 Tests	Complete Test Kit

[IVD]

Intended Use

Prolactin (PRL) is a polypeptide hormone consisting of a single polypeptide chain of approx. 200 amino acids. The hormone is secreted from the anterior pituitary. Its major physiological action is on the mammary gland, where PRL stimulates growth, induces and maintains milk production. Pathologically increased PRL levels have been reported in cases of prolactin-producing adenomas of the pituitary, leading to clinical symptoms such as amenorrhoea, hypogonadism and galactorrhoea. The clinical and diagnostic usefulness of PRL measurement for ascertaining the diagnosis of hyperprolactinemia and for subsequent monitoring of treatment efficiency has been well established.

Principle - Direct Antigen EIA -

The HUMAN PRL ELISA is intended for professional use. The ELISA for direct antigen detection uses the high affinity of biotin for streptavidin, which has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and enzyme conjugate (peroxidase-labeled monoclonal anti-PRL and biotinylated monoclonal anti-PRL) are mixed to form a specific immunocomplex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin. At the end of the incubation excess enzyme conjugate and monoclonal antibodies are washed out. TMB/Substrate is added (step 2) and the resulting colour, which turns into yellow after stopping the reaction with the stop solution, is measured photometrically. The intensity of the colour is directly proportional to the PRL concentration in the sample.

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). The concentration is evaluated by means of a calibration curve which is established from the calibrators supplied with the kit.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with streptavidin	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, light yellow PRL level: 0 (A), 5 (B), 10 (C), 25 (D), 50 (E), and 100 (F) ng/ml	
[CON]	13 ml	Antibody-Enzyme Conjugate (white cap) ready for use, light orange anti-PRL (monoclonal, mouse), HRP-labeled, anti-PRL (monoclonal, mouse), biotinylated	1.0 µg/ml
[WS]	20 ml	Wash Solution (black cap) Concentrate for approx. 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) ~~Urea~~ Hydrogen peroxide Sodium acetate buffer	1.0 g/l ~~0.03%~~ 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulfuric acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS], will completely dissolve on dilution.
- Dilute [WS] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	25	--
Specimens, Controls; in duplicate	--	25
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

Validation of the Test

The test results are valid, if the following criteria are met:

The mean absorbance (OD) of [CAL] F ≥ 1.3.

The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

PRL shows a noticeable circadian rhythm, being elevated during sleep. It is therefore recommended that samples are taken 2 hours after sleep or in the early morning after a 12 hours fasting period. Uptake of morphine and reserpine as well as treatment with psychotropic and hypertensive drugs has to be carefully assessed as these drugs may elevate PRL secretion.

Stress and mild exercise may cause a moderate increase of PRL concentrations. Since PRL concentrations depend on diverse factors other than pituitary homeostasis, the clinical status should not be assessed on PRL determination alone.

Human anti-mouse antibodies (HAMA) may show either falsely elevated or depressed values in patients treated with mouse monoclonal antibodies.

Pregnancy, lactation, and oral contraception can increase the PRL levels.

Expected Values

Basal values	PRL [ng/ml]
Women, non-pregnant	1.2 – 19.5
Women, postmenopausal	1.5- 18.5
Men	1.8- 17.0

Values above 200 ng/ml PRL are indicative for pituitary adenomas, such above 250 ng/ml PRL confirm a tumor process.

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The PRL ELISA test has an analytic sensitivity of approx. 0.8 ng/ml PRL.

Patient samples with abnormally high PRL levels may cause a hook effect (paradoxical low O.D.s). If this is suspected dilute specimen 1/100 with [CAL] A (0.0 ng/ml PRL) re-assay and multiply the result by 100. Values up to 3000 ng/ml still gave absorbances greater than the [CAL] F.

The assay is standardised in accordance with WHO 3rd International Standard for PRL 1988 (84/500).

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-prl.pdf or

www.human-de.com/data/gb/vr/el-prl.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

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EL-PRL INF 5303001 GB 08-2014-23M

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fPSA

ELISA Test for the Quantitative Determination of free Prostate Specific Antigen in Human Serum

Package Size

[REF]	52035	96 Tests	Complete Test Kit

[IVD]

Intended Use

Prostate Specific Antigen (PSA) is a glycoprotein (serine protease) with a molecular weight of 28.4 kDa[1] synthesised by epithelial cells of the prostate. PSA is not only detected in men but also in women with mamma carcinoma (30-40%). PSA levels in men are found to be elevated in prostatitis, benign prostate hyperplasia (BPH) and malignant or metastatic prostate cancer (PCA). Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and therapeutic monitoring of patients due to increased sensitivity.[2]

In human serum PSA occurs in two forms, the free and the complexed molecule which can be detected by immunological methods. Free PSA (fPSA) is determined by this ELISA.

The fPSA fraction was shown to be substantially smaller in patients with untreated PCA than in patients with BPH.[3] Therefore determination of total PSA (tPSA) and fPSA may lead to a better discrimination between BPH and PCA.

Principle - Direct Antigen EIA -

The HUMAN fPSA ELISA is intended for professional use. The ELISA for direct antigen detection uses the high affinity of Biotin for Streptavidin, which has been coated on the surface of microtiter wells. In the first incubation step, specimens, calibrators or controls and antibody-enzyme conjugate (biotinylated monoclonal anti-fPSA antibody and peroxidase-labelled monoclonal anti-fPSA antibody) are mixed to form a specific immunocomplex which is bound to the surface of the wells by the interaction of biotin with the immobilised streptavidin. At the end of the incubation excess conjugate and unbound antigen are washed out. TMB/Substrate is added, a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the fPSA concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with streptavidin	
[CAL]	A – F 6x2.0ml	Calibrators (white cap, coloured labels: A: white, B: yellow, C: green, D: red, E: blue, F: black) ready for use, in human serum, yellowish fPSA level: 0 (A), 0.5 (B), 1.0 (C), 2.5 (D), 5.0 (E) and 10.0 (F) ng/ml	
[CON]	13 ml	Antibody-Enzyme Conjugate (white cap) ready for use, coloured green anti-fPSA antibody (monoclonal, mouse), HRP-labelled anti-fPSA antibody (monoclonal, mouse)	
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for approx. 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	< 0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- sealed in an aluminium bag with a desiccant.
- before opening, the strips must be at room temperature.
- unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Specimen should be obtained prior to clinical intervention [10].

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: <15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	50	--
Specimens, Controls; in duplicate	--	50
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line) at 450 nm. Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known fPSA concentrations.

Validation of the Test

The test results are valid, if the following criteria are met:

The mean absorbance (OD) of [CAL] F ≥ 1.3.

The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

In the blood PSA is mostly complexed by the protease inhibitor anti-chymotrypsin (ACT).[4] This PSA-ACT complex is typically the major form in the circulation of prostate cancer patients (PCA), it accounts for about 85% of the total PSA (tPSA) present. 12-15% of PCA patients show fPSA as the predominant form. [5]

A number of studies found that the fPSA is much more increased in patients with benign prostatic hyperplasia (BPH) than in patients with PCA[5]. Therefore it has been proposed to perform a combined interpretation of the tPSA particularly for the range of 4 to 10 ng/ml tPSA with the ratio of fPSA to tPSA to improve diagnosis respectively differentiation of PCA and BPH.[6,7,8]

These figures may significantly vary, if tests from different sources are used for determination of tPSA and fPSA, due to possible variations of the respective antibody specificities. The above figures are only valid for the combination of HUMAN PSA ELISA and HUMAN fPSA ELISA.

Limitations

Hormonal therapy for prostate cancer may alter PSA expression. Therefore a low PSA result following a prostatic cancer treatment may not precisely reflect the presence of residual tissue or recurrent disease.[11] All relevant patient history and clinical data must be considered before making any critical decision.

Expected Values

	fPSA level (n = 122)
Healthy male, with normal prostate (≤ 4ng/ml tPSA)	≤ 0.8 ng/ml

The probability for PCA will be increased, if the tPSA is higher than 4 ng/ml and the percentage ratio of fPSA/tPSA is less than 25%.[7,8,9]

fPSA / tPSA	Probability of PCA
≤ 10%	55%
10-15%	28%
15-20%	25%
> 20%	10%

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The HUMAN fPSA ELISA test has an analytical sensitivity of 0.05 ng/ml specimen.

Specimens with fPSA concentrations higher than 10 ng/ml should be diluted (1+9) with normal serum ([CAL] A) and re-assayed. Multiply the result by 10.

The assay is standardised in accordance with the 1st IS issued by WHO No. 96/668.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-fpsa.pdf or

www.human-de.com/data/gb/vr/el-fpsa.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

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PSA

ELISA Test for the Quantitative Determination of total Prostate Specific Antigen in Human Serum

Package Size

[REF]	52030	96 Tests	Complete Test Kit
[IVD]			

Intended Use

Prostate Specific Antigen (PSA) is a glycoprotein (serine protease) with a molecular weight of 28.4 kDa[1] synthesised by epithelial cells of the prostate. PSA is not only detected in men but also in women with mamma carcinoma (30-40%). In male serum PSA can be detected as free or complex molecule by immunological methods[2]. Total PSA is detected by this ELISA.

Elevated PSA levels are found in benign hyperplasia, prostatitis as well as in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphate (PAP) in the diagnosis and therapeutic monitoring of patients due to increased sensitivity[3].

Principle - Direct Antigen EIA -

The HUMAN PSA ELISA is intended for professional use. The ELISA for direct antigen makes use of matched highly specific monoclonal anti-PSA antibodies coated on the surface of microtiter wells, and covalently linked to enzyme respectively. In the first incubation step, specimens, calibrators or controls and antibody-enzyme conjugate are mixed to form the sandwich complex on the surface of the wells. At the end of the incubation excess conjugate and unbound antigen are washed out. TMB/Substrate is added (step 2), and a blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the PSA concentration in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with anti-PSA (monoclonal, mouse)	
[CAL]	A - F	Calibrators – Coloured caps and labels (A: white, B: yellow, C: green, D: red, E: blue, F: black)	
	6x2.0ml	Ready to use, in serum matrix PSA level: 0 (A), 2.5 (B), 5.0 (C), 10 (D), 25 (E), and 50 (F) ng/ml	
[CON]	13 ml	Antibody-Enzyme Conjugate (white cap) ready to use, coloured red anti-PSA (mab, mouse), HRP-labelled	
[WS]20x] 5102	50 ml	Wash Solution (white cap) Concentrate for ca. 1000 ml	
		Tris-Buffer	10 mmol/l
		NaCl	8 g/l
[SUB] 5103	13 ml	Substrate Reagent (black cap) Ready to use, colourless to bluish	
		3,3', 5,5'-tetramethylbenzidine (TMB)	1.2 mmol/l
		Hydrogen Peroxide	≤ 6.0 mmol/l
[STOP] 5104	15 ml	Stop solution (red cap)	
		Sulphuric acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.1%.

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/630–690 nm filters, deionised water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The general purpose reagents [WS]20x] 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits.

Working Wash Solution [WASH]

- Dilute [WS]20x] 1 + 19 with fresh deionised water, e.g. 50 ml [WS]20x] + 950 ml = 1000 ml.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Specimen should be obtained prior to clinical intervention [4].

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9: [MIC] - rock gently for 20-30 sec. after each pipetting step without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker (e.g. HUMAN's HumaReader line).

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing 4 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	25	--
Specimens, Controls; in duplicate	--	25
[CON]	100	100
Mix and cover [MIC] with Adhesive Strip		
Incubate 30 min at 20...25°C		
Wash 5 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Incubate 15 min at 20...25°C (see P8)		
[STOP]	100	100
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 10 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of unknown specimen is calculated from a dose response curve generated by utilising calibrators of known PSA concentrations.

Readers and automated analyzers

Validated settings for HUMAN ELISA microplate readers (HumaReader) or automated HUMAN ELISA analyzers (ELISYS line) are preinstalled or can be obtained from your local distributor. Application sheets for Human instruments with analyzer/assay specific handling and performance information are accessible via: www.human.de/aps-elisa.

For automated analyzers other than those provided by HUMAN follow section Pipetting Scheme and ensure all requirements described in section Procedural Notes are followed. All protocols for automated analyzers must be fully validated prior to usage.

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] F ≥ 1.2.
- The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

Prostate cancer, the second most prevalent form of male malignancy, shows a strong increase in incidence in male with the age of 50.

The most sensitive method for diagnostic and therapeutic monitoring is the quantitative determination of PSA in serum. The PSA concentration has a good correlation to size and developmental status of the tumour.

PSA levels are elevated not only in malign carcinoma, but also in benign prostate hyperplasia (BPH). Therefore determination of the PSA value alone is not sufficient to assess cancer. It should be used in conjunction with other clinical manifestations and diagnostic parameters (e.g. digital rectal examination, sonography of prostate)[5, 6].

High levels of PSA decrease very fast after surgical prostate removal. Persistent high levels usually indicate residual malignant areas, or metastasis.

An increase of PSA values at a conservative therapy indicates the progression of prostate cancer in an early state (until 6 months earlier than other diagnostic methods).

Free PSA determinations may be helpful in regard to the discrimination of BPH and prostate cancer conditions [7].

Limitations

Hormonal therapy for prostate cancer may alter PSA expression. Therefore a low PSA result following a prostatic cancer treatment may not precisely reflect the presence of residual tissue or recurrent disease [8]. All relevant patient history and clinical data must be considered before making any critical decision.

Expected Values

	PSA level
Healthy male, with normal prostate	< 4 ng/ml

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The HUMAN PSA ELISA test has an analytical sensitivity of 0.1 ng PSA /ml.

Specimens with PSA concentrations above 50 ng/ml should be diluted (1+9) with a confirmed PSA negative serum. Multiply the result by 10.—([CAL] A is not recommended for sample dilution.)

The assay is standardised in accordance with the 1st IS issued by WHO No. 96/668.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-psa.pdf or
www.human-de.com/data/gb/vr/el-psa.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Safety Notes

[STOP] Warning

~~- Hazard statements~~

H315 Causes skin irritation.

H319 Causes serious eye irritation.

~~[SUB] **Danger**~~

~~- Hazard statements~~

~~H360D May damage the unborn child.~~

~~- Precautionary statements~~

[CAL] [CON] [WS20x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV 1/2 antibodies and HBsAg and found negative.~~

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

References

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SLE Latex Test

Latex Agglutination Slide Test for the Detection of Antinuclear Antibodies Associated with Systemic Lupus Erythematosus (SLE)

Package Sizes

[REF]	~~40031~~	~~25 Tests~~	~~Complete Test Kit~~
	40030	50 Tests	Complete Test Kit

[IVD]

Intended Use

Systemic Lupus Erythematosus (SLE) is classed as the prototype of severe autoimmune disease, involving a variety of tissues and associated with a wide range of antibodies in the circulation. In SLE, mostly antinuclear antibodies (ANA) are detected. They are directed against desoxyribonucleoprotein (DNP) and several other extractable nuclear constituents[1,2], as well as native deoxyribonucleic acid (n-DNA). The organs affected are, in decreasing incidence, joints, skin, kidney, central nervous system, heart and lungs.

ANA may be IgG, IgM or IgA found in about 95% of active untreated SLE patients. As indicated under Notes the ANA are not completely specific for the SLE[1, 2], but for the diseases mentioned the titers are substantially lower than in SLE[3, 4]. Antibodies to DNP detected by the HUMAN assays are the most common ANA involved in SLE. They were found in more than 90% of patients with SLE[7]. This assay is intended for professional use and the analysis should be performed by trained laboratory personnel only.

Principle

The SLE LATEX TEST provides a means of detecting anti-DNP in human serum. SLE Latex Reagent is a stabilized buffered suspension of polystyrene latex particles that have been coated with DNP. When the Latex Reagent is mixed with the serum containing antibodies to DNP, agglutination occurs. Using dilutions of reactive patient sample, the anti-DNP titer can be determined.

Contents, Reagents, Composition

[LR]	~~CE 25 or 50~~ SLE Latex Reagent (black and white cap)	
	Suspension of polystyrene latex particles, coated with desoxyribonucleoprotein (calf thymus).	
	Sodium azide	0.095 %
[PC]	0.5 ml Positive Control (red cap)	
	Liquid control, ready for use, containing desoxyribonucleoprotein antibodies (human) sufficient to give a distinct agglutination	
	Sodium azide	0.095 %
[NC]	1.0 ml Negative Control (green cap)	
	Liquid control, ready for use, non reactive with [LR]	
	Sodium azide	0.095 %
	1 Slide with 6 cells	

Stability

[LR], [PC] and [NC] are stable up to the stated expiry date when stored at 2...8°C in an upright position.

Do not freeze reagents!

Specimen

Use fresh serum.

Storage up to 48 hours at 2...8°C.

Procedure

Bring [LR], [PC], [NC] and sera to room temperature and mix latex reagent gently before use. Do not heat reagents in a water bath.

Expel the contents of the [LR] dropper, then refill. Use one drop of reagent for each serum specimen and each control.

A. Qualitative Test

Place in separate cells of the slide.			
	Serum	[PC]	[NC]
Serum	30 µl	---	---
[PC]	---	1 drop	---
[NC]	---	---	1 drop
[LR]	1 drop	1 drop	1 drop
Mix with separate sticks and spread the fluid over the entire area of the cells.			
Gently tilt and rotate the slide for one (1) minute and observe for agglutination.			

(1 drop ≈40 µl)

B. Semiquantitative Test

Dilute 250 µl specimen with 250 µl NaCl (9 g/l) (Dilution no. 1) and proceed further according to the following scheme:						
Dil. no.	1	2	3	4	5	6
Transfer		250 µl 1	250 µl 2	250 µl 3	250 µl 4	250 µl 5
NaCl		250 µl	250 µl	250 µl	250 µl	250 µl
Titer	1/2	1/4	1/8	1/16	1/32	1/64
Continue test as described under A employing each dilution as specimen.						

Interpretation, Quality Control

A. Qualitative Test

[PC] and [NC] have to be run with each series and compared with the unknown specimens to distinguish possible granularity from agglutination.

The test is considered as negative when no difference in agglutination is observed between specimen and [NC].

[PC] and positive sera must show distinct agglutination within 1 minute.

Agglutination indicates the level of antinuclear antibody (specifically, anti-DNP) in the range commonly found in SLE.

B. Semiquantitative Test

The titer of antinuclear antibodies (anti-DNP) is the reciprocal of the highest dilution which exhibits a positive reaction.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/lx-sle.pdf or

www.human-de.com/data/gb/vr/lx-sle.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Notes

1. Serum from patients with scleroderma, rheumatoid arthritis, dermatomyositis, and a variety of connective tissue diseases may elicit agglutination in the SLE slide test. [3, 4].
2. Titer changes in sequential samples from the same patient may or may not correlate with the activity of the disease.
3. Use clean, dry slides which have previously been rinsed with distilled water.
4. In accord with all diagnostic methods, a final diagnosis should not be made on the result of a single test, but should be based on a correlation of test results with other clinical findings.
5. Store all reagents in an upright position when not in use.

Safety Notes

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/ national/ international regulations.

All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be negative using approved methods. However, the material should still be regarded as potentially infectious.

References

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T3

ELISA Test for the Quantitative Determination of Total Triiodothyronine (T3) in Human Serum or Plasma

Package Size

[REF]	54010	96 Tests	Complete Test Kit
[IVD]			

Intended Use

Triiodothyronine (T3) is a hormone synthesised and stored in the thyroid gland. More than 99% of T3 in the blood is bound reversibly to plasma proteins. The concentration of T3 is much lower than that of T4, but its metabolic potency is much greater. T3 determination is an important tool in thyroid disease diagnosis. Its measurement has uncovered a variant of hyperthyroidism where thyrotoxic patients present elevated T3 values with normal T4 values (T3-hyperthyroidism). An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. The clinical significance of T3 is also evident in patients in whom euthyroidism is attributable only to normal T3, though their T4 values are subnormal. T3 determination is also useful in monitoring both patients under treatment for hyperthyroidism and patients who have discontinued antithyroid drug therapy. It is especially valuable in distinguishing between euthyroid and hyperthyroid subjects. In addition to hyperthyroidism, T3 levels increase during pregnancy, oral contraception or estrogen treatment, paralleling TBG (Thyroxine Binding Globulin) increases in a manner analogous to T4. Likewise, a decrease in TBG concentration decreases T3 concentration. These changes in the T3 level, however, are not a true reflection of thyroid status. Best diagnostic information about the thyrostasis in such situations can be obtained by the TRH test.

Principle - Competitive EIA -

The HUMAN T3 ELISA is intended for professional use. The ELISA is based on the principle of competitive binding between T3 in a test specimen and T3-peroxidase conjugate for a limited number of binding sites on the anti-T3 (sheep) coated well. Thus the amount of T3-peroxidase conjugate bound to the well is inversely proportional to the concentration of T3 in the specimen.

After incubation of specimen and T3-peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. TMB/Substrate solution is added (step 2), and a blue colour develops. The intensity of this colour, which changes to yellow after stopping the reaction, is inversely proportional to the amount of T3 in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with anti-T3 (sheep)	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, yellowish T3 level: 0 (A), 0.5 (B), 1.0 (C), 2.5 (D), 5.0 (E), and 7.5 (F) ng/ml	
[CON]	1.5 ml	Enzyme-antigen Conjugate (white cap) T3-HRP-conjugate, coloured yellow In a protein stabilising matrix	1 %
[C-DIL]	13 ml	Conjugate buffer (white cap) Tris buffered saline, coloured red	
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for approx. 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	< 0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working conjugate solution [WCON]

- Dilute [CON] 1 + 10 with [C-DIL]: e.g. dilute 160 µl [CON] with 1.6 ml [C-DIL] for 16 wells.
- Stability: 24 h at 2...8°C

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum or plasma (EDTA, Heparin)

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	50	--
Specimens, Controls; in duplicate	--	50
[WCON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Specimen's concentration is extrapolated from a dose response curve generated by utilising serum calibrators of known antigen concentrations

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] A ≥ 1.3.
- The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, TBG concentration, and the binding of triiodothyronine to TBG.[3,4]

Thus, total triiodothyronine concentration alone is not sufficient to assess the clinical status.

Total serum triiodothyronine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A decrease in T3 values is found with protein-wasting diseases, certain liver diseases and administration of hormones and drugs.[3]

Expected Values

Results from a study with euthyroid subjects:

Mean (X)	1.36 ng/ml
Standard Deviation (S.D.)	0.33 ng/ml
Expected Ranges (± 2 S.D.)	0.69 - 2.02 ng/ml

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory as the T3 levels are much influenced by geographical and dietary factors

Performance Characteristics

The T3 ELISA test has an analytic sensitivity of approx. 0.05 ng/ml T3.

Specimens with T3 concentrations higher than 7.5 ng/ml may be diluted with [CAL] A and re-assayed. To obtain the sample's concentration multiply by the dilution factor.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-t3.pdf or

www.human-de.com/data/gb/vr/el-t3.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

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T4

ELISA Test for the Quantitative Determination of Total Thyroxine (T4) in Human Serum or Plasma

Package Size

[REF]	54020	96 Tests	Complete Test Kit

[IVD]

Intended Use

L-Thyroxine (T4) is a hormone synthesised and stored in the thyroid gland. More than 99% of T4 in the blood is bound reversibly to plasma proteins (mainly Thyroxine Binding Globulin, TBG).

Measurement of total T4 by immunoassay technique is one of the most reliable and convenient screening tests available to determine the presence of thyroid disorders. Elevated levels of T4 have been found in hyperthyroidism due to Grave's disease and Plummer's disease as well as in acute and subacute thyroiditis. Low levels of T4 have been associated with cretinism, myxedema, Hashimoto's disease and with some genetic abnormalities.

Principle - Competitive EIA -

The HUMAN T4 ELISA is intended for professional use. The ELISA is based on the principle of competitive binding between T4 in a test specimen and T4-peroxidase conjugate for a limited number of binding sites on the anti-T4 (sheep) coated well. Thus the amount of T4-peroxidase conjugate bound to the well is inversely proportional to the concentration of T4 in the specimen.

After incubation of specimen and T4-peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. TMB/Substrate solution is added (step 2) and a blue colour develops. The intensity of this colour, which changes to yellow after stopping the reaction, is inversely proportional to the amount of T4 in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with anti-T4 (sheep)	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, in human serum, yellowish T4 level: 0 (A), 2 (B), 5 (C), 10 (D), 15 (E), and 25 (F) µg/dl	
[CON]	1.5 ml	Enzyme-antigen Conjugate (white cap) T4-HRP-conjugate, coloured yellow In a protein stabilising matrix	1 %
[C-DIL]	13 ml	Conjugate buffer (white cap) Tris buffered saline, coloured red	
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for approx. 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	< 0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric acid	0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working conjugate solution [WCON]

- Dilute [CON] 1 + 10 with [C-DIL]: e.g. dilute 160 µl [CON] with 1.6 ml [C-DIL] for 16 wells.
- Stability: 24 h at 2...8°C

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum or plasma (EDTA, Heparin)

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	25	--
Specimens, Controls; in duplicate	--	25
[WCON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Specimen's concentration is interpolated from a dose response curve generated by utilising serum calibrators of known antigen concentrations.

Validation of the Test

The test results are valid, if the following criteria are met:

- The mean absorbance (OD) of [CAL] A ≥ 1.3.
- The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

Total serum thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG.[3,4] Thus, total thyroxine concentration alone is not sufficient to assess the clinical status.

Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A decrease in T4 values is found with protein-wasting diseases, certain liver diseases and administration of hormones and drugs[3]. Best diagnostic information about the thyrostasis in such situations can be obtained by the TRH test.

Expected Values

	Male	Female
Mean (X)	7.6 µg/dl	8.2 µg/dl
Standard deviation (S.D.)	1.6 µg/dl	1.7 µg/dl
Expected range (± 2 S.D.)	4.4 - 10.8 µg/dl	4.8 - 11.6 µg/dl

Normal patients with high TBG levels were not excluded except if pregnant.

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory as the T4 levels are much influenced by geographical and dietary factors

Performance Characteristics

The T4 ELISA test has a analytic sensitivity of about 0.22 µg/dl T4.

Specimens with T4 concentrations higher than 25 µg/dl may be diluted with [CAL] A and re-assayed. To obtain the sample's concentration multiply by the dilution factor.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-t4.pdf or

www.human-de.com/data/gb/vr/el-t4.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

1. Barker S.B., Journal Biological Chemistry 173, 175 (1948)
2. Chopra I.J. *et al.*, J. Clinical Endocrinol. 33, 865 (1971)
3. Young D.S. *et al.*, Clinical Chemistry 21, 3660 (1975)
4. Sterling L., Cleveland CRC Press p. 19 - 51 (1975)

EL-T4 INF 5402001 GB 07-2015-26M

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++++ Change of ¼ ++++ Please read marked text carefully! ++++

Testosterone

ELISA Test for the Quantitative Determination of Testosterone in Human Serum or Plasma

Package Size

[REF]	55010	96 Tests	Complete Test Kit

[IVD]

Intended Use

Testosterone (17-beta-Hydroxy-4-androstene-3-on) is a C_{19} steroid with a molecular weight of 288 Dalton.

It is the most important male sex hormone. In men it is primarily synthesised in the testes by the cells of Leydig and in women by the ovaries (25%), adrenal cortex (25%) and by peripheral conversion of androstendione (50%). About 98% of the secreted hormone circulating in the bloodstream is bound to beta-globulin. The remaining portion (free testosterone) is supposed to be the active steroid.

Determination of testosterone gives an indication for the function of testes or ovaries. Low levels of testosterone result in hypogonadism. It's clinical picture is directly related to the age of development of hormone deficiency. Related disorders are syndromes like Klinefelter's or Turner's and cryptorchism or anorchia, but also the female climacteric periode and meonopause.

Hypergonadism, due to increased testosterone values, is detected in male and female patients with an androgen producing tumour of testes, ovaries or adrenal cortex. Increased testosterone levels in women may confirm hirsutism, virilisation or polycystic ovary syndrome.[1,2]

Principle - Competitive EIA -

The HUMAN TESTOSTERONE ELISA is intended for professional use. The ELISA is based on competitive interaction of testosterone and the hormone-enzyme conjugate for a limited number of immobilised monoclonal anti-testosterone antibodies (mouse). Thus, the amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of testosterone in the specimen.

After incubation of specimen and hormone-enzyme conjugate in the well, unbound conjugate is removed by washing. When substrate solution is added (step 2), a blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is inversely proportional to the amount of testosterone in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in strip holder) 8-well snap-off strips, coated with anti-testosterone antibodies (mouse)	
[CAL]	A - G 7x1 ml	Calibrators (white cap) ready for use, in human serum, yellowish Testosterone level: 0 (A), 0.2 (B), 0.5 (C), 1.0 (D), 2.0 (E), 6.0 (F) and 16.0 (G) ng/ml	
[CON]	25 ml	Enzyme Conjugate (white cap) Ready for use, coloured red Testosterone – HRP – conjugate BSA TRIS/MOPS buffer NaCl	 pH 6.9 ± 0.2 0.5 % 0.05 mol/l 0.1 mol/l
[WS]40x]	30 ml	Wash Solution (black cap) concentrate for about 1200 ml Tween 20 TRIS buffered saline	 pH 7.0 ± 0.1 0.5 % 3.0 mol/l
[SUB]	25 ml	Substrate Reagent (yellow cap) Ready for use 3,3', 5,5'-tetramethylbenzidin (TMB) Hydrogen peroxide Sodium acetate buffer DMSO	pH 3.5 – 4.0 0.26 g/l 0.015 % 0.05 mol/l < 5 %
[STOP]	14 ml	Stop Solution (red cap) sulphuric acid, ready for use	 0.5 mol/l

Preservatives: Total concentration < 0.7%

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/~~630-690~~620 – 630 nm filters, destilled ~~deionised~~ water.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within ~~60 days~~8 weeks.

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used for 60 days.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Dilute [WS]40x] to 1200 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 2 weeks, stored at 15...25°C.

Specimen

Serum or ~~EDTA~~ plasma (EDTA, citrate, heparin)

Do not use highly haemolytic, icteric or lipaemic specimens and samples containing sodium azide.

Specimens may be stored for ~~3~~7 days at 2...8°C, up to ~~30 days~~ 12 months at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration dates.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9: [MIC] - rock gently for 20-30 sec. after each pipetting step without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker.

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 3 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...F2 Calibrators	G2... Specimen
[CAL] A-G; in duplicate	25	--
Specimens, Controls; in duplicate	--	25
[CON]	200	200
Mix		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	400	400
Step 2		
[SUB]	200	200
Incubate 15 min at 20...25°C (see P8)		
[STOP]	100	100
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within **3**0 min. after terminating of reaction, using a reference wavelength of ~~630-690~~620 – 630 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Concentration of unknown specimen is interpolated from a dose response curve generated by utilising serum calibrators of known testosterone concentrations.

Validation of the Test

The test results are valid provided the following criteria are met:

- The mean absorbance (OD) of [CAL] A ≥ 1.0.
- The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points results in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

The results have to be interpreted on the background that the concentration of testosterone shows cyclic variations. Short intensive exertions may result in an increase, long exhausting work in a decrease of the serum concentration[3]. Also, heavy illnesses (e.g. of liver, kidney or circulation), stress, anaesthesia, drugs (heroin, methadone) and medicines (e.g. antimycotica) may decrease its concentration.

Values < 3 ng/ml in fresh morning serum are an indication of hypogonadism for adult men. The incidence for hypognadism will increase with the age of male patients.

Androgenism of women caused by polycystic ovaries may be indicated by values of 0.6 - 1.5 ng/ml. Cancer of female patients is suspected at > 1.5 ng/ml.

Determination of testosterone and DHEA-S provides the opportunity for differentiation of androgen producing tumours from other diseases by testing of sera from ovary and adrenal cortex.[4]

Determination of the testosterone value alone is not sufficient for diagnosis of pathological conditions. It should be used in conjunction with other clinical manifestations and diagnostic (hormone) parameters.

Expected Values

	Testosterone level
Sexually mature women	< 0.6 ng/ml
Postmenopause	< 0.8 ng/ml
Boys before puberty	0.3 – 1.2 ng/ml
Adult men	3.5 – 8.6 ng/ml

Conversion factor: 1 ng/ml = 3.47 nmol/l

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The TESTOSTERONE ELISA test has an analytic sensitivity of 0.05 – 0.09 ng/ml specimen.

Specimens with testosterone concentrations above 16 ng/ml should be diluted (1+9) with [CAL] A (0 ng/ml) and re-assayed. Multiply the result by 10.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-testo.pdf or

www.human-de.com/data/gb/vr/el-testo.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

Safety Notes

[STOP] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

[WS]40x] Warning

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

· Precautionary statements

[CAL] [CON] [WS]40x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.

References

1. Kicklighter E.J., Norman R.J., Clin. Chem. 43, 658-660 (1989)
2. Schwartz U. *et al.*, J. Gynecol. 14, 119-130 (1981)
3. Schürmeyeer T., Nieschlag E., Int. J. Androl. 7, 276-282 (1984)
4. Moltz L. *et al.*, Fertil. Steril. 42, 69-75 (1984)

EL-Testo INF 5501001 GB 07-2020-025

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TSH

ELISA Test for the Quantitative Determination of Thyrotropin (TSH) in Human Serum

Package Size

[REF]	54030	96 Tests	Complete Test Kit

[IVD]

Intended Use

Thyrotropin (TSH) is a glycoprotein hormone of approx. 28 kDa, secreted from the anterior pituitary gland. It is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism[1,2]. Increase in serum concentrations of TSH is an early and sensitive indicator of decreased thyroid reserve and in conjunction with decreased T4 is diagnostic of primary hypothyroidism. The expected increase in TSH demonstrates the classical negative feedback system between the pituitary and thyroid glands. In addition, TSH determination is useful in differentiating secondary and tertiary hypothyroidism from the primary thyroid disease. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal.

Principle - Direct Antigen-EIA -

The HUMAN TSH ELISA is intended for professional use. As a 2nd generation assay, the TSH ELISA makes use of a highly specific monoclonal anti-TSH antibody coated on the surface of the microtiter wells. In the first incubation step, specimens, calibrators or controls and enzyme conjugate (peroxidase-labelled anti-TSH) are mixed to form the sandwich complex which is bound to the surface of the wells by the interaction with the immobilised antibody. At the end of the incubation excess enzyme conjugate is washed out. Substrate reagent is added (step 2) and the resulting colour, which turns into yellow after stopping the reaction with the stop solution, is measured photometrically. The intensity of colour is directly proportional to the TSH concentration in the sample.

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). The concentration is evaluated by means of a calibration curve which is established from the calibrators supplied with the kit.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with anti-TSH (monoclonal, mouse)	
[CAL]	A - F	Calibrators – Coloured caps and labels (A: white, B: yellow, C: green, D: red, E: blue, F: black)	
	6x2.0ml	Ready to use, (human) TSH level: 0 (A), 0.5 (B), 3.0 (C), 6.0 (D), 15.0 (E), and 30.0 (F) mIU/l	
[CON]	13 ml	Enzyme Conjugate (white cap) ready to use, coloured red anti-TSH (goat), HRP-labelled	pH 6.25 ± 0.1
[WS]20x 5102	50 ml	Wash Solution (white cap) Concentrate for ca. 1000 ml Tris-Buffer NaCl	 pH 7.2 ± 0.2 10 mmol/l 8 g/l
[SUB] 5103	13 ml	Substrate Reagent (black cap) Ready to use, colourless to bluish 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen Peroxide	pH 3.6 ± 0.25 1.2 mmol/l ≤ 6.0 mmol/l
[STOP] 5104	15 ml	Stop solution (red cap) Sulphuric acid	 0.5 mol/l
	1	Adhesive strip	

Preservatives: Total concentration < 0.1%.

Additional materials recommended but not supplied with the kit

Micropipettes, ELISA washer, microplate reader equipped with 450 nm or with 450/630–690 nm filters, deionised water

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days.

[MIC] (Code: TSH)

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date.
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The general-purpose reagents [WS]20x 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits.

Working Wash Solution [WASH]

- Dilute [WS]20x 1 + 19 with fresh deionised water, e.g. 50 ml [WS]20x + 950 ml = 1000 ml.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9: [MIC] - rock gently for 20-30 sec. after each pipetting step without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker (e.g. HumaReader).

P10: Always firmly close vials with the proper caps after use.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing 4 times.

W2: In case of automatic washers fill and prime with [WASH]. Subsequently wash strips 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	50	--
Specimens, Controls; in duplicate	--	50
[CON]	100	100
Mix and cover [MIC] with Adhesive Strip		
Incubate 60 min. at 20...25°C		
Wash 5 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Incubate 15 min. at 20...25°C (see P8)		
[STOP]	100	100
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

Readers and automated analyzers

Validated settings for HUMAN ELISA microplate readers (HumaReader) or automated HUMAN ELISA analysers (ELISYS line) are preinstalled or can be obtained from your local distributor. Application sheets for Human instruments with analyser/assay specific handling and performance information are accessible via: www.human.de/aps-elisa.

For automated analysers other than those provided by HUMAN follow section Pipetting Scheme and ensure all requirements described in section Procedural Notes are followed. All protocols for automated analysers must be fully validated prior to usage.

Validation of the Test

The test results are valid, if the following criteria are met:

The mean absorbance (OD) of [CAL] F ≥ 1.2.

The difference between the duplicates of [CAL] F does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points results in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. Thus, thyrotropin concentration alone is not sufficient to assess clinical status. TSH may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodine, phenobarbital, and phenytoin have been reported to increase TSH levels. A decrease in TSH has been reported with the administration of propranolol, methimazol, dopamine and D-thyroxine. Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result.

Expected Values

Reference values from euthyroid population:

Normal range: 0.3 - 4.0 mIU/l TSH

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory.

Performance Characteristics

The TSH ELISA test as a 2nd generation assay has an analytical sensitivity of < 0.10 mIU/l TSH and can therefore distinguish hyperthyroid from euthyroid population.

Specimens with TSH concentrations higher than 30 mIU/l should be diluted 1+9 with [CAL] A or 0.9% NaCl and re-assayed. The concentration of TSH determined for the diluted sample must be multiplied by the dilution factor to obtain the actual concentration of TSH in the specimen.

The assay is standardised in accordance with the WHO 3rd IS for human TSH for immunoassay (NIBSC code 81/565).

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-tsh.pdf or

www.human-de.com/data/gb/vr/el-tsh.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

Safety Notes

[STOP] Warning

~~- Hazard statements~~

H315 Causes skin irritation.

H319 Causes serious eye irritation.

~~- Precautionary statements~~

~~[SUB] Danger~~

~~- Hazard statements~~

~~H360D May damage the unborn child.~~

~~- Precautionary statements~~

[CAL] [CON] [WS][20x] [SUB] [STOP]

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with

water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

~~The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative.~~

All patient specimens, calibrators and controls should be handled as potentially infectious. All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be non-reactive using approved methods. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of human or animal origin should still be treated as potentially infectious material.

References

1. Barker S. B., Journal Biological Chemistry 173, 175 (1948)
2. Chopra I. J. *et al.*, J. Clinical Endocrinol. 33, 865 (1971)
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EL-TSH INF 5403H01 GB 10-2020-36

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fT3

ELISA Test for the Quantitative Determination of Free Triiodothyronine (fT3) in Human Serum

Package Size

[REF]	54015	96 Tests	Complete Test Kit

[IVD]

Intended Use

Triiodothyronine (T3) is a hormone formed in the thyroid gland by peripheral deiodination of thyroxine (T4). Both, T3 and T4, are secreted into the circulation in response to thyroid stimulating hormone (TSH). They play an important role in the regulation of metabolism.

Most of the secreted T3 (99.7%) is reversibly bound to transport proteins i.e. thyroxine-binding globulin (TBG) and to a much lesser extent albumin and prealbumin.[1,2] The remaining unbound part of T3 circulates free (fT3) in the blood stream. It is supposed to be the biologically active component. fT3 is much more active than fT4.

fT3 levels correlate to the secretion of T3 and to its metabolism. However, changes in T3 binding proteins (TBG) result in altered levels of total T3 while fT3 levels remain constant. Under such conditions determination of fT3 is useful. It reflects more reliably the clinical status than determination of total T3.

In normal healthy individuals TBG levels are relatively stable. However under certain clinical conditions such as pregnancy or steroid therapy TBG levels are altered. Therefore determination of fT3 is indicated, which is also the case for identification of all forms of hyperthyroidism and monitoring of patients on anti-thyroid therapy.

Principle - Competitive EIA -

The HUMAN fT3 ELISA is intended for professional use. The ELISA is based on the principle of competitive binding between fT3 in a test specimen and T3-peroxidase conjugate for a limited number of binding sites on the anti-T3 (sheep) coated well. Thus the amount of T3-peroxidase conjugate bound to the well is inversely proportional to the concentration of fT3 in the specimen.

After incubation of specimen and T3-peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. TMB/Substrate solution is added (step 2), and a blue colour develops. The intensity of this colour, which changes to yellow after stopping the reaction, is inversely proportional to the amount of fT3 in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with anti-T3 (sheep)	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, yellowish in human serum, at approximate* concentrations of fT3: 0 (A), 1 (B), 3 (C), 5 (D), 8 (E), and 16 (F) pg/ml	
[CON]	13 ml	Enzyme-Antigen Conjugate (white cap) ready for use, coloured red T3-HRP conjugate in a protein Stabilising matrix	 1 %
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for ca. 1000 ml Tris buffered saline	 250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Sodium acetate buffer Hydrogen peroxide	 < 0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop solution (red cap) Sulphuric acid	 0.5 mol/l
	1	Adhesive strip	

* Exact levels are given on the labels on a lot specific basis

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes.

All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found to be negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices. All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	50	--
Specimens, Controls; in duplicate	--	50
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wavelength of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Specimen's concentration is extrapolated from a dose response curve generated by utilising serum calibrators of known antigen concentrations.

Validation of the Test

The test results are valid, if the following criteria are met:

The mean absorbance (OD) of [CAL] A ≥ 1.3.

The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

fT3 is independent of changes in the concentrations and binding properties of binding proteins. Additional determination of a binding parameter (T-uptake, TBG) is therefore not necessary.

- Dilution of samples with high concentrations (absorbances below [CAL] F) is not recommended! TBG variations in different matrices will not allow fT3 hormone to dilute serially.
- Circulating autoantibodies to fT3 and hormone-binding inhibitors may interfere[3].
- Heparin may affect fT3 levels in vivo and in vitro. Collect samples before patients undergo anticoagulant treatment[4].
- Extreme variations in albumin binding capacity for T3 (rare conditions) may result in misinterpretation of fT3.

This assay is not evaluated for new-born screening!

For diagnostic purposes, the fT3 results should be assessed in conjunction with patient's history, clinical examination and other findings.

Expected Values

	Adult	Pregnant
Mean (X)	2.8 pg/ml	3.0 pg/ml
Standard deviation (S.D.)	0.7 pg/ml	0.6 pg/ml
Expected range (± 2 S.D.)	1.4 – 4.2 pg/ml	1.8 – 4.2 pg/ml

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory as the fT3 levels are much influenced by geographical and dietary factors.

Performance Characteristics

The fT3 ELISA test has an analytic sensitivity of about 0.5 pg/ml fT3.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-ft3.pdf or

www.human-de.com/data/gb/vr/el-ft3.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

References

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EL-FT3 INF 5401501 GB 07-2015-14M

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fT4

ELISA Test for the Quantitative Determination of Free Thyroxine (fT4) in Human Serum

Package Size

[REF]	54025	96 Tests	Complete Test Kit
[IVD]			

Intended Use

L-Thyroxine (T4) is the most important thyroid hormone. It is secreted like T3 into the circulation in response to thyroid stimulating hormone (TSH). Both, T3 and T4, play an important role in the regulation of metabolism.

T4 circulates in blood almost completely bound (> 99.9%) to carrier proteins.[1] The main carrier is the thyroxine-binding globulin (TBG). The free (unbound) fraction of thyroxine (fT4) is supposed to be the biologically active component.

In many clinical conditions (e. g. pregnancy) with regular thyroid function concentrations of the binding proteins are altered also affecting total T4 concentration whereas fT4 level remains constant. Also oral contraceptives, estrogen therapy, drugs[2] and hormone binding inhibitors[3] may result in abnormal total T4 levels while the fT4 levels remain in the normal range. The fT4 concentration correlates much better with the clinical status than the total thyroxine level.

Determination of fT4 is indicated for both hyper- and hypothyroid conditions. It uncovers the patient's actual clinical status because changes in TBG concentration may result in a normal T4 level masking the abnormal thyroid function.

Principle - Competitive EIA -

The HUMAN fT4 ELISA is intended for professional use. The ELISA is based on the principle of competitive binding between fT4 in a test specimen and T4-peroxidase conjugate for a limited number of binding sites on the anti-T4 (monoclonal, mouse) coated well. Thus, the amount of T4-peroxidase conjugate bound to the well is inversely proportional to the concentration of fT4 in the specimen.

After incubation of specimen and T4-peroxidase conjugate unbound enzyme conjugate is removed in the equilibrium state by washing. TMB/substrate solution is added (step 2), and a blue colour develops. The intensity of this colour, which changes to yellow after stopping the reaction, is inversely proportional to the amount of fT4 in the specimen.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, coated with anti-T4 (monoclonal, mouse)	
[CAL]	A - F 6x2.0ml	Calibrators (white cap) ready for use, yellowish, in human serum, at approximate* concentrations of fT4: 0 (A), 0.40 (B), 1.25 (C), 2.10 (D), 5.00 (E), and 7.40 (F) ng/dl	
[CON]	13 ml	Enzyme-Antigen Conjugate (white cap) ready for use, coloured green T4-HRP conjugate in a protein stabilising matrix	1 %
[WS]50x]	20 ml	Wash Solution (black cap) Concentrate for ca. 1000 ml Tris buffered saline	250 mmol/l
[SUB]	14 ml	Substrate Reagent (yellow cap, ready for use) 3,3', 5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide Sodium acetate buffer	< 0.25 g/l 0.03 mol/l
[STOP]	7.5 ml	Stop Solution (red cap) Sulphuric acid	0.5 mol/l
	1	Adhesive strip	

* Exact levels are given on the labels on a lot specific basis

Preservatives: Total concentration < 0.04%.

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and [CAL] should be handled as potentially infectious. [CAL] have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or [CAL] should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

~~[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.~~

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be stored at 2...8°C and used within 60 days (see also "Note").

[MIC]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Working Wash Solution [WASH]

- Faint turbidity, which may appear in the concentrate [WS]50x], will completely dissolve on dilution.
- Dilute [WS]50x] to 1000 ml with fresh, deionised water in a suitable container. Rinse vial several times.
- Stability: up to 60 days at 15...25°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 5 days at 2...8°C, up to 30 days at -20°C. Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record [CAL], specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number and place firmly in the holder.

P5: Run duplicates for [CAL], controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 10 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove adhesive strips, aspirate off the contents, add [WASH], aspirate off after 30 sec. soak time and repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...D2 Calibrators	E2... Specimen
[CAL] A-F; in duplicate	50	--
Specimens, Controls; in duplicate	--	50
[CON]	100	100
Rock gently and cover [MIC] with Adhesive Strip		
Incubate 60 min at 20...25°C		
Wash 3 times as described (see W1 - W3)		
[WASH]	300	300
Step 2		
[SUB]	100	100
Do not shake [MIC] after [SUB] addition		
Incubate 15 min at 20...25°C (see P8)		
[STOP]	50	50
Mix carefully		
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of reaction, using a reference wave-length of 630-690 nm (if available).		

The absorbance of calibrators and specimen is determined by using ELISA microplate readers or automated ELISA systems (like HUMAN's HumaReader or ELISYS line). Specimen's concentration is extrapolated from a dose response curve generated by utilising serum calibrators of known antigen concentrations.

Validation of the Test

The test results are valid, if the following criteria are met:

The mean absorbance (OD) of [CAL] A ≥ 1.3.

The difference between the duplicates of [CAL] A does not exceed 10%.

Calculation

Plot measured absorbances against [CAL] concentrations in a lin-lin graph. Appropriate interpolation of plotted measuring points result in a calibration curve, from which the analyte concentration in the sample can be determined.

For calculation of analyte concentrations select an appropriate and validated curve fitting option (recommendation: point to point).

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of Results

fT4 is independent of changes in the concentrations and binding properties of binding proteins. Additional determination of a binding parameter (T-uptake, TBG) is therefore not necessary.

- Dilution of samples with high concentrations (absorbances below [CAL] F) is not recommended! TBG variations in different matrices will not allow fT4 hormone to dilute serially.
- Heparin may affect fT4 levels in vivo and in vitro. Collect samples before patients undergo anticoagulant treatment.[4]
- Circulating autoantibodies to fT4, hormone-binding inhibitors and high concentrations of rheumatoid factors may interfere.[5, 6]
- Extreme variations in albumin binding capacity for T4 (rare conditions, see familial dysalbuminemic hyperthyroxinemia, FDH) may result in misinterpretation of fT4.[7]

This assay is not evaluated for newborn screening!

For clinical diagnosis, the fT4 findings should be assessed in conjunction with the patient's history, clinical examination and other findings because fT4 concentration is dependent on a multiplicity of factors including drugs affecting binding capacity of carrier proteins. [8,9]

Expected Values

	Adult	Pregnant
Mean (X)	1.4 ng/dl	1.5 ng/dl
Standard deviation (S.D.)	0.3 ng/dl	0.37 ng/dl
Expected range (± 2 S.D.)	0.8 – 2.0 ng/dl	0.8 – 2.2 ng/dl

Each laboratory should establish its own Expected Values utilising instrumentation, blood collection methods and testing techniques commonly used in that laboratory as the fT4 levels are much influenced by geographical and dietary factors.

Performance Characteristics

The fT4 ELISA test has an analytic sensitivity of about 0.05 ng/dl fT4.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-ft4.pdf or

www.human-de.com/data/gb/vr/el-ft4.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The components of the kit are stable until the expiry date even after opening. However, a potential contamination is directly related to the number of samplings. The 60 days limit after first use is set for safety reasons.

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

[STOP] Warning!

· Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

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25-OH Vitamin D

ELISA for the quantitative measurement of 25-OH Vitamin D in human serum.

Package Size

[REF]	55500	96 Tests	Complete Test kit
[IVD]			

Intended Use

The 25-OH Vitamin D ELISA is used for the quantitative determination of 25-hydroxy Vitamin D2 and D3 (25-OH-D2 and 25-OH-D3) in human serum.

Vitamin D is the generic term used to designate Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). 25-OH Vitamin D stimulates the intestinal absorption of both calcium and phosphate and also bone resorption and mineralisation. Vitamin D deficiency is an important risk factor for rickets, osteomalacia, senile osteoporosis, cancer, pregnancy outcomes and autoimmune diseases. The measurement of both 25-OH Vitamin D forms is also required to determine the cause of abnormal serum calcium concentrations in patients. Vitamin D intoxication has been shown to cause kidney and tissue damages.

Principle

The 25-OH Vitamin D ELISA is a solid phase enzyme-linked immunosorbent assay performed on microtiter plates for professional use. During a first incubation step at room temperature, total 25-OH Vitamin D (D2 and D3) present in calibrators, controls and samples is dissociated from its binding serum proteins to a specific monoclonal antibody (mAb). After a washing step, a defined amount of biotin-labelled 25-OH Vitamin D in presence of streptavidin-horseradish peroxidase (HRP), compete with 25-OH Vitamin D2 and 25-OH Vitamin D3 bound to the paratopes of the specific monoclonal antibody. After further incubation at room temperature, the microtiter plate is washed to stop the competition reaction. Now the substrate is added and incubated. The colour-forming reaction is stopped by addition of stop solution, the colour turns from blue to yellow. Then the microtiterplate is read at the appropriate wavelength (450 nm). The amount of formed reaction product is determined colourimetrically by measuring the absorbance, which is inversely proportional to the total 25-OH Vitamin D (D2 and D3) concentration. A calibration curve is plotted and the total 25-OH Vitamin D (D2 and D3) concentrations of the samples are determined by interpolation from the calibration curve.

Reagents and Contents

[MTP]	12	Microtiter Strips (in strip holder) 8-well snap-off strips, coated with anti-(25-OH Vitamin D2 and D3) mAb
[CAL]0]	~~2~~ 1 ml	Vitamin D Calibrator, 0 ng/ml (white cap, grey label), lyophilized
[CAL]1]-[CAL]5]	1 ml	Vitamin D Calibrators (white cap, yellow label), lyophilized For calibrator levels please refer to the bottle label
[CONTROL]1]	1 ml	Vitamin D Control 1 (white cap, red label) Lyophilized, human plasma For control levels please refer to the bottle label
[CONTROL]2]	1 ml	Vitamin D Control 2 (white cap, red label) Lyophilized, human plasma For control levels please refer to the bottle label
[INC]BUF]	20 ml	Incubation Buffer (green cap) Ready for use
[CON]100x]	300 µl	Vitamin D Conjugate Solution (white cap) Concentrate (100x), dilute with [CON]BUF]
[CON]BUF]	30 ml	Conjugate Buffer (white cap), Ready for use
[HRP]200x]	200 µl	HRP Solution (yellow cap), Concentrate (200x), dilute with [CON]BUF]
[WASH]200x]	10 ml	Washing buffer (brown cap) Concentrate (200x) Tris buffer pH 8.0
[SUB]	12 ml	Substrate, TMB solution (brown bottle) Ready for use 3,3',5,5'-tetramethylbenzidine
[STOP]	12 ml	Stop Solution (red cap), Ready for use HCl 1M

Additional material required (not provided)

1. Vortex mixer (e.g. HumaTwist*)
2. Magnetic stirrer
3. Plate shaker (400 rpm)
4. Washer for microtiter plates (e.g. Combiwash*)
5. Microtiter plate reader (e.g. HumaReader*) or automated instruments (e.g. Elisys Line*) capable of reading at 450 nm and 650 nm (bichromatic reading)

*material available from HUMAN

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

Stability

Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the label, if kept at 2...8°C.

[MTP]

- Sealed in an aluminium bag with a desiccant.
- Before opening, the strips must be at room temperature.
- Unused: return to the zip-lock bag with the desiccant. Strips stored in this way at 2...8°C can be used until the expiration date (see also "Note").
- Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Mix all reagents and samples thoroughly by gentle agitation or swirling.

Working Wash Solution **[WASH]**

- Dilute 1 part [WASH]200x] with 199 parts distilled water, use a clean plastic container. Use a magnetic stirrer to mix thoroughly.
- Freshly prepared working wash solution should be used on the same day. Alterations in physical appearance of kit reagents may indicate instability or deterioration.

Calibrator and Control Working Solutions

- ~~[CAL]0]: Reconstitute the calibrator 0 with 2 ml distilled water~~
- [CAL]0] - [CAL]5]: Reconstitute the calibrators ~~1~~ 0 - 5 with 1 ml distilled water.
- [CONTROL]1] + [CONTROL]2]: Reconstitute the controls with 1 ml distilled water.
- After reconstitution, calibrators and controls are stable for 8 weeks at 2…8°C. For longer storage periods, aliquots should be stored at –20°C for maximum 4 months. Avoid subsequent freeze-thaw cycles.

Working Conjugate Solution

š[HRP]: The working HRP conjugate solution is to be prepared during the incubation and minimum 45 minutes before its use.

Prepare an adequate volume of [HRP] by mixing the 3 reagents in the following sequence:

(1) [CON]BUF], (2) [CON]100x], (3) Vortex, (4) [HRP]200x], (5) Vortex.

š The order of addition of those 3 reagents is critical and should be rigorously respected to get reproducible optical densities.

Prepare [HRP] according to the number of used strips, as indicated in the table below.

For example for 6 strips (48 wells):

60 µl [CON]100x] and 30 µl [HRP]200x] to 6 ml [CON]BUF]. Use a Vortex mixer to homogenize.

Until its use, keep [HRP] at room temperature and avoid direct sunlight or use a brown glass vial for its preparation. The preparation of working [HRP] is not stable and must be discarded if not used.

Number of strips	Volume (µl) [CON]100x]	Volume (µl) [HRP]200x]	Volume (ml) [CON]BUF]
1	10	5	1
2	20	10	2
3	30	15	3
4	40	20	4
5	50	25	5
6	60	30	6
7	70	35	7
8	80	40	8
9	90	45	9
10	100	50	10
11	110	55	11
12	120	60	12

Specimen

This kit is suitable for serum and plasma heparinised samples.

Serum and heparinised plasma provide similar results

If the test is not run after collection within 24 hrs, storage at -20°C is recommended. Avoid subsequent freeze-thaw cycles.

Hemolysed samples should not be used.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix or use components with different lot numbers. Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record calibrators, specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MTP] - select the required number and place firmly in the holder.

P5: Run duplicates for calibrators, controls and specimens. Pipette them on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 20 minutes.

P7: Avoid/remove air bubbles prior to incubations and reading absorbance.

P8: [SUB] initiates and [STOP] terminates a kinetic reaction. Avoid bright light during colour development.

P9: [MTP] - rock gently during incubation (400 rpm) without spilling the solutions to ensure thorough mixing. If available mix on a plate shaker.

P10: Pipettes – use clean disposable pipette tips for addition of each reagent and sample. Avoid pipettes with metal parts for dispension of [SUB] and [STOP].

P11: Prepare calibration curve for each run, do not use data from previous runs.

P12: Respect the incubation times.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Aspirate off the contents, add [WASH] and aspirate off again. Repeat washing twice.

W2: In case of automatic washers prime with [WASH] and wash strips 3 times additionally. Ensure the washer fills all wells completely and aspirates off efficiently (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.		
Step 1	*Well [µl]*	
	A1...F2 Calibrators	G1... Controls, Specimen
[CAL]0], [CAL]1] to [CAL]5], in duplicate	25	-
Specimens, Controls; in duplicate	-	25
[INC]BUF]	75	75
Incubate 60 min. at 20...25°C on a shaker, see P9		
Prepare the [HRP] during the sample incubation (time required min 45 min.)		
Wash 3 times as described (see W1 - W3)		
[WASH]	350	350
Step 2		
[HRP]	100	100
Incubate 15 min. at 20...25°C on a shaker, see P9		
Wash 3 times as described (see W1 - W3)		
[WASH]	350	350
Step 3 (within 15 min after W3)		
[SUB]	100	100
Incubate 15 min. at 20...25°C on a shaker, see P9		
[STOP]	100	100
Measure the absorbance at 450 nm (reference filter 630 nm or 650 nm) within 60 min.		

Validation of the test

[CONTROL]1] and [CONTROL]2] are within the range specified on the vial label.

Calculation of results

- Calculate the mean of duplicate determinations.
- The use of computer-assisted methods to construct the calibration curve is recommended. 4-parameter logistic function curve fitting is the preferred method.
- By interpolation of the OD values, determine the 25-OH Vitamin D concentrations of the samples from the calibration curve.
- Samples suspected of containing concentrations above the highest calibrator should be assayed in dilution by [CAL]0].

Quality Control

Good laboratory practice requires controls to be assayed with each calibration curve. A statistically significant number of controls should be run to establish mean values and acceptable ranges to assure proper performance. Quality control samples should be run according to local regulations. The results should be within the established ranges.

Interpretation of the results

Dietary intake, origin, season, and age are known to affect the normal levels of 25-OH Vitamin D.

25 OH Vitamin D status	Expected values
Deficient	<10 ng/ml
Insufficient	10 - 29 ng/ml
Sufficient	30 - 100 ng/ml
Potential Toxicity	>100 ng/ml

Each laboratory should establish its own range based on their local population.

The test is an aid in the diagnosis and is to be used in conjunction with clinical findings.

The performance of this assay has not been established in a pediatric population.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/el-55500-2.pdf or

www.human-de.com/data/gb/vr/el-55500-2.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The handling should always be in compliance with common good laboratory practice requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions as patient specimens etc. / Stock solutions always have to be returned to 2...8°C when not in use.)

Safety Notes

[INC]BUF] Danger

· Hazard statements

H360D-H362 May damage the unborn child. May cause harm to breast-fed children.

H371 May cause damage to organs.

· Precautionary statements

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P263 Avoid contact during pregnancy/while nursing.

P280 Wear protective gloves/protective clothing/eye protection/face protection

P264 Wash thoroughly after handling.

P270 Do not eat, drink or smoke when using this protocol.

P201 Obtain special instructions before use.

P202 Do not handle until all safety precautions have been read and understood.

P308+P313 If exposed or concerned: Get medical advice/attention.

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

[STOP] Warning

· Hazard statements

H290 May be corrosive to metals.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

· Precautionary statements

P234 Keep only in original container.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P264 Wash thoroughly after handling.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P302+P352 IF ON SKIN: Wash with plenty of water.

P362+P364 Take off contaminated clothing and wash it before reuse.

P406 Store in corrosive resistant container with a resistant inner liner.

References

1. Zerwek J.E., Blood biomarkers of Vitamin D status, Am. J. Clin. Nutr., 87, 1087S-91S (2008)
2. Heaney R.P., Defining Deficiency of Vitamin D, Clinical Laboratory Internat, 34, 16-19 (2010)
3. Holick M.F., Vitamin D Status: Measurement, Interpretation, and Clinical Application, Ann. Epidemiol., 19, 73-78 (2009)
4. Holick M.F., Vitamin D Deficiency. N. Engl. J. Med., 357, 266-281 (2007)

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