Elecsys Anti-Tg

cobas®

REF		Σ	SYSTEM
09005021190* 09005021214*	09005021500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
ATG	10202

Please note

The measured anti-Tg value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the anti-Tg assay method used. Anti-Tg values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the anti-Tg assay procedure used while monitoring therapy, then the anti-Tg values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

Immunoassay for the in vitro quantitative determination of antibodies to thyroglobulin in human serum and plasma. The anti-Tg determination is used as an aid in the detection of autoimmune thyroid diseases.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Thyroglobulin (Tg) is produced in the thyroid gland and is a main component in the lumen of the thyroid follicle. In synergy with the enzyme thyroid-specific peroxidase (TPO), Tg has an essential function in the iodination of L-tyrosine and in the formation of the thyroid hormones T4 and T3.[1] Both Tg and TPO are potentially autoantigenic.[2,3]

Elevated serum concentrations of antibodies against Tg (Tg-autoantibodies) are found in subjects with autoimmunity-based thyroiditis.[2,3] High concentrations of anti-Tg together with anti-TPO are present in most patients with chronic lymphocytic-infiltrative thyroiditis (Hashimoto's disease).[3] The frequency of thyroglobulin antibodies is approximately 50-80 % in subjects with autoimmune-thyroiditis, including Hashimoto's disease, and approximately 30-50 % in individuals with Graves' disease.[3,4,5,6] The anti-Tg assay can also provide useful information for monitoring the course of Hashimoto's thyroiditis and for differential diagnosis.[3,7] This includes cases of suspected autoimmune thyroiditis of unknown origin with negative anti-TPO test results,[8,9] and to distinguish Hashimoto's thyroiditis from nontoxic nodular goiter or from other forms of thyroiditis.[4]

Anti-Tg has also been reported as a useful surrogate diagnostic marker for differentiated thyroid cancer when serum Tg is negative,[10] and for ruling out interference by Tg autoantibodies when measuring serum Tg using a Tg test.[11,12]

Although the sensitivity of the procedure can be increased by simultaneously determining additional thyroid antibodies (anti-TPO, anti-TSHR), a negative result does not definitively rule out the presence of an autoimmune disease. The antibody titer does not correlate with the clinical activity of the disease. Titers that are elevated initially can become negative if the disease persists for a longer period of time or if remission occurs. If antibodies reappear after remission, relapse is likely.

The Elecsys Anti-Tg assay uses human antigen and monoclonal human anti-Tg antibodies.[13]

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 µL of sample are incubated with biotinylated Tg and the antibodies of the sample bind the antigen.
- 2nd incubation: After addition of anti-Tg antibodies labeled with ruthenium complex[a)] and streptavidin-coated microparticles, the immunocomplex produced becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as ATG.

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Tg~biotin, 1 bottle, 19.7 mL:
Biotinylated Tg (human) 0.200 mg/L; TRIS buffer 100 mmol/L, pH 7.0; preservative.

R2 Anti-Tg-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 19.7 mL:
Monoclonal anti-Tg antibodies (human) labeled with ruthenium complex 0.620 mg/L; TRIS buffer 100 mmol/L, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

Digitally signed by Lazari Cristina
Date: 2024.12.13 12:22:40 EET
Reason: MoldSign Signature
Location: Moldova

Elecsys Anti-Tg

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P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A). However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[14,15]

The initial thyroid glandular tissue extract containing the human thyroglobulin has shown to be free from HBsAg and antibodies to HCV and HIV.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 20 IU/mL + coefficient of correlation ≥ 0.95.

Stable for 4 days at 20-25 °C, 4 days at 2-8 °C, 2 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 09005030190, Anti-Tg CalSet, for 4 x 1.5 mL
- REF 05042666191, PreciControl ThyroAB, for 4 x 2.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the NIBSC (National Institute for Biological Standards and Control) 65/93 Standard.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

Use Elecsys PreciControl ThyroAB or other suitable controls for routine quality control procedures.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in IU/mL or kIU/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL

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Compound	Concentration tested
Hemoglobin	≤ 0.373 mmol/L or ≤ 600 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 300 IU/mL

Criterion: For concentrations of 10-75 IU/mL the deviation is ≤ 11 IU/mL. For concentrations > 75 IU/mL the deviation is ≤ 15 %.

For samples ≤ 115 IU/mL no interference was oberserved for hemoglobin concentrations ≤ 600 mg/dL. In samples with a concentration of > 115 IU/mL a lower hemoglobin concentration may result in increased anti-Tg values.

Pharmaceutical substances
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested mg/L
Iodide	50
Carbimazole	30
Methimazole	16
Propylthiouracil	180
Perchlorate	2000
Propranolol	48
Amiodarone	40
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.300
Levothyroxine	0.250
Liothyronine	0.045
Nivolumab	96

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

For Tg concentrations exceeding the normal range (> 100 ng/mL) an influence on anti-Tg concentrations of more than 15 % may occur.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

10-4000 IU/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 10 IU/mL. Values above the measuring range are reported as > 4000 IU/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 9 IU/mL

Limit of Detection = 10 IU/mL

Limit of Quantitation = 15 IU/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Sample dilution is not possible. The autoantibodies are heterogeneous and this gives rise to non-linear dilution phenomena.

Approximately 5 % of the pathological samples can have concentrations ≥ 4000 IU/mL.

Expected values

Studies conducted with the Elecsys Anti-Tg assay in 5 clinical centers covering a total of 391 healthy subjects (MCE Elecsys Anti-Tg assay) confirmed the threshold value of 115 IU/mL; this value corresponds to the 94th percentile.

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", English: REF 04640292.

This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults. Different inclusion and exclusion criteria were applied (e.g. sonographic results (thyroid volume and density) as well as criteria according to the guidelines of the National Academy of Clinical Biochemistry - NACB).

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	14.2	0.639	4.5	1.12	7.9
Human serum 2	114	2.67	2.3	3.50	3.1
Human serum 3	1676	37.7	2.3	50.2	3.0
Human serum 4	1985	46.8	2.4	58.6	3.0
Human serum 5	3378	90.1	2.7	110	3.3
PC[b)] THYRO1	63.6	1.58	2.5	2.60	4.1
PC THYRO2	148	3.72	2.5	4.68	3.2

b) PC = PreciControl

Method comparison

a) A comparison of the Elecsys Anti-Tg assay, REF 09005021190 (**cobas e** 801 analyzer; y), with the Elecsys Anti-Tg assay, REF 07026919190 (**cobas e** 801 analyzer; x), gave the following correlations (IU/mL):

Number of samples measured: 150

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Passing/Bablok[16]	Linear regression
y = 1.01x - 5.04	y = 1.03x - 27.9
τ = 0.949	r = 0.997

The sample concentrations were between 10.3 and 3785 IU/mL.

b) A comparison of the Elecsys Anti-Tg assay, REF 09005021190 (**cobas e** 402 analyzer; y), with the Elecsys Anti-Tg assay, REF 09005021190 (**cobas e** 801 analyzer; x), gave the following correlations (IU/mL):

Number of samples measured: 161

Passing/Bablok[16]	Linear regression
y = 1.03x - 0.385	y = 1.05x - 7.00
τ = 0.972	r = 0.999

The sample concentrations were between 10.0 and 3675 IU/mL.

Analytical specificity

The following cross-reactivities were tested with anti-Tg concentrations of approximately 30 IU/mL and 115 IU/mL.

No influence with human autoantibodies to thyroid peroxidase (< 1500 IU/mL) was detectable.

References

1 Mansourian AR. Metabolic pathways of tetraidothyronine and triidothyronine production by thyroid gland: a review of articles. Pak J Biol Sci 2011;14(1):1-12.

2 Ruf J, Ferrand M, Durand-Gorde JM, et al. Significance of thyroglobulin antibodies cross-reactive with thyroperoxidase (TGPO antibodies) in individual patients and immunized mice. Clin Exp Immunol 1993;92(1):65-72.

3 Thomas L. Thyroid function. Thyroglobulin antibodies. In: Thomas L (ed.). Deutsch: Labor und Diagnose. TH-Books, Frankfurt. 5th edition 1998:1043. English: Clinical Laboratory Diagnosis. 1st edition 1998:1021.

4 Slatosky J, Shipton B, Wahba H. Thyroiditis: differential diagnosis and management. Am Fam Physician 2000;61(4):1047-1052.

5 Garber JR, Cobin RH, Gharib H, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and The American Thyroid Association. Thyroid 2012;22(12):1200-1235.

6 Iddah MA, Macharia BN. Autoimmune thyroid disorders. ISRN Endocrinol 2013:509764.

7 Schmeltz LR, Blevins TC, Aronoff SL, et al. Anatabine supplementation decreases thyroglobulin antibodies in patients with chronic lymphocytic autoimmune (Hashimoto's) thyroiditis: A randomized controlled clinical trial. J Clin Endocrinol Metab 2014;99:E137-E142.

8 Feldt-Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. Clin Chem 1996;42(1):160-163.

9 Lazarus J, Brown RS, Daumerie C, et al. 2014 European Thyroid Association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Eur Thyroid J 2014;3:76-94.

10 Nam HY, Paeng JC, Chung JK, et al. Monitoring differentiated thyroid cancer patients with negative serum thyroglobulin. Diagnostic implication of TSH-stimulated antithyroglobulin antibody. Nuklearmedizin 2014;53(2):32-38.

11 Spencer CA, Takeuchi M, Kazarosyan M, et al. Serum Thyroglobulin Antibodies: Prevalence, Influence on Serum Thyroglobulin Measurement, and Prognostic Significance in Patients with Differentiated Thyroid Carcinoma. J Clin Endocrin Metabol 1998;83(4):1121-1127.

12 Spencer C. International Thyroid Testing Guidelines. National Academy of Clinical Biochemistry, August 2001;Section 3E,11-14.

13 Prentice L, Kiso Y, Fukuma N, et al. Monoclonal Thyroglobulin Autoantibodies: Variable Region Analysis and Epitope Recognition. J Clin Endocrin Metabol 1995;80:977.

14 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

15 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

16 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Elecsys CA 15-3 II

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REF		Σ	SYSTEM
07027001190* 07027001214*	07027001500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
CA 15-3 2	10002

Please note

The measured CA 15-3 value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the CA 15-3 assay method used. CA 15-3 values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the CA 15-3 assay procedure used while monitoring therapy, then the CA 15-3 values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

Immunological in vitro assay for quantitative determination of CA 15-3 in human serum and plasma to aid in the management of breast cancer patients. In conjunction with other clinical and diagnostic procedures, serial testing with this assay is an aid

- in the early detection of recurrence in previously treated stage II and III breast cancer patients
- for monitoring response to therapy in metastatic breast cancer patients

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

The CA 15-3 (Cancer Antigen 15-3) is derived from glycoprotein Mucin-1 (MUC-1).[1] The CA 15-3 assay uses two monoclonal antibodies (MAb), 115D8 and DF3, in a sandwich assay to detect two antigenic sites associated with breast carcinoma cells. MAb 115D8 is directed against human milk fat globule membranes,[1,2,3] whereas MAb DF3 is directed against the membrane fraction from human breast cancer.[4]

The antigen is normally found in the luminal secretion of glandular cells and does not circulate in the blood. When cells become malignant and their basal membranes permeable, the antigen is detectable in serum. [5] Overexpression of MUC1 plays an important role in epithelial to mesenchymal transition; an important and complex phenomenon that determines cancer progression.[6] The guideline landscape for advanced disease monitoring was mapped in a review by Duffy et al.[7]
The low cost and minimally invasive CA 15-3 monitoring approach is mentioned in ASCO and the European Group on Tumor Markers (EGTM) guidelines, especially if there is non-measurable disease in conventional imaging.[8] The ESMO breast cancer guidelines suggest that tumour markers such as CA 15-3 may be useful to evaluate response to treatment, particularly in patients with non-measureable metastatic disease. A change in tumour markers alone should not be used to initiate a change in treatment.[7]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 6 µL of sample are automatically prediluted 1:20 with Diluent Universal. The antigen (in 12 µL of prediluted sample), a biotinylated monoclonal CA 15-3-specific antibody, and a monoclonal CA 15-3-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as CA15-3 2.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-CA 15-3-Ab~biotin, 1 bottle, 19.7 mL:
Biotinylated monoclonal antibody (115D8; mouse) 1.75 mg/L; phosphate buffer 20 mmol/L, pH 6.0; preservative.

R2 Anti-CA 15-3-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 19.7 mL:
Monoclonal anti-CA 15-3 antibody (DF3; mouse) labeled with ruthenium complex 10 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Elecsys CA 15-3 II

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Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 2 U/mL + coefficient of correlation ≥ 0.95.

Stable for 48 hours at 20-25 °C, 5 days at 2-8 °C, 90 days at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03045846122, CA 15-3 II CalSet, 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the Elecsys CA 15-3 assay. This in turn has been standardized against the Enzymun-Test CA 15-3 method and CA 15-3 RIA by Fujirebio Diagnostics.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in U/mL or kU/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70 ng/mL
Rheumatoid factors	≤ 1500 IU/mL

Criterion: Recovery ± 1.5 U/mL of intial value for samples ≤ 15 U/mL, within ± 10 % of initial value for samples > 15-50 U/mL, and within ± 13 % of initial value for samples > 50 U/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

Typically, no high-dose hook effect[b)] can be observed at CA 15-3 concentrations up to 20000 U/mL. However, due to the heterogeneous nature of the CA 15-3 antigen a high-dose hook effect below this value

cannot be completely excluded. In case of an unexpected low result, the sample should be diluted 1:10 (refer to Section "Dilution") and retested.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special cancer drugs were tested. No interference with the assay was found.

Special cancer drugs

Drug	Concentration tested µg/mL
Carboplatin	200
Cisplatin	225
Cyclophosphamide	1000
Doxorubicin	75
Etoposide	400
5-FU	500
Flutamide	1000
Methotrexate	200
Mitomycin	25
Tamoxifen	50
Taxol	5.5

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

b) High-dose hook effect: A sample with a true concentration clearly above the measuring range, but found within the measuring range.

Limits and ranges

Measuring range

1.5-300 U/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 1.5 U/mL. Values above the measuring range are reported as > 300 U/mL (or up to 3000 U/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 1.0 U/mL

Limit of Detection = 1.5 U/mL

Limit of Quantitation = 3 U/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

An internal study was performed based on guidance from the CLSI, protocol EP17-A2. Limit of Blank and Limit of Detection were determined to be the following:

Limit of Blank = 0.576 U/mL

Limit of Detection = 1.10 U/mL

For Limit of Quantitation ≥ 4 human serum samples were measured over 5 days with 5 replicates per day on one analyzer. With an intermediate precision CV of ≤ 20 %, the Limit of Quantitation was 1.60 U/mL.

Dilution

Use Diluent Universal for automatic sample predilution. Samples with CA 15-3 concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:10 (either automatically by the analyzer or manually). The concentration of the diluted sample must be > 30 U/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

• *Healthy subjects:*

Results of a reference range study using a panel of samples from 374 apparently healthy non-pregnant females (Roche study No. RD000788)

Percentile (%)	U/mL	Confidence interval (U/mL)
95	26.2	25.2-27.9
97.5	28.5	26.7-34.5
99	34.5	28.7-57.8

• *Patients with benign diseases and pregnant women:*

Relative distribution of CA 15-3 concentrations in patients with benign disease and pregnant women (Roche study No. B00P018)

	Subjects total	< 25 U/mL	25-50 U/mL	> 50-200 U/mL	> 200 U/mL
	N	Classification in percent (%)			
Gastrointestinal	109	84	16	0	0
Breast	58	88	12	0	0
Gynecological diseases	42	83	12	5	0
Renal failure	37	81	19	0	0
Urological diseases	34	82	18	0	0
Bacterial infection	27	96	4	0	0
Pregnancy	34	97	0	3	0

• *Patients with malignant diseases (others than breast):*

Relative distribution of CA 15-3 concentrations in individuals with malignancy other than breast

	Subjects total	< 25 U/mL	25-50 U/mL	> 50-200 U/mL	> 200 U/mL
	N	Classification in percent (%)			
Stomach-Ca[c)]	36	75	14	8	3
Hepatocellular-Ca	37	59	32	3	5
Lung-Ca	38	82	13	5	0
Ovarian-Ca	34	47	21	29	3
Gynecological-Ca	5	40	20	40	0
Prostate-Ca	48	79	17	4	0
Colorectal-Ca	40	93	8	0	0
Pancreatic-Ca	40	65	33	3	0

c) Ca = Carcinoma

• *Patients with breast cancer:*

Relative distribution of CA 15-3 concentrations in patients with breast malignancy. The staging of patients according to UICC criteria was

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performed at primary diagnosis before any treatment. The patients diagnosed with recurrent disease had developed metastases (M1).

	Subjects total	< 25 U/mL	25-50 U/mL	> 50-200 U/mL	> 200 U/mL
	N	Classification in percent (%)			
UICC I	56	88	12	0	0
UICC II	126	85	13	2	0
UICC III	77	53	30	14	3
UICC IV	24	25	17	37	21
Recurrent Disease	75	15	25	36	24

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	2.51	0.110	4.4	0.305	12.2
Human serum 2	26.7	0.528	2.0	0.768	2.9
Human serum 3	130	3.73	2.9	4.45	3.4
Human serum 4	157	7.03	4.5	7.95	5.0
Human serum 5	280	4.07	1.5	8.24	2.9
PC[d)] Tumor Marker1	23.2	0.300	1.3	0.621	2.7
PC Tumor Marker2	95.7	1.49	1.6	2.88	3.0

d) PC = PreciControl

Method comparison

A comparison of the Elecsys CA 15-3 II assay, REF 07027001190 (**cobas e** 801 analyzer; y) with the Elecsys CA 15-3 II assay, REF 03045838122 (**cobas e** 601 analyzer; x) gave the following correlations (U/mL):

Number of serum samples measured: 198

Passing/Bablok[9]	Linear regression
y = 0.994x - 0.065	y = 0.978x + 0.686
τ = 0.969	r = 0.998

The sample concentrations were between 5.14 and 279 U/mL.

A comparison of the Elecsys CA 15-3 II, REF 07027001190 (**cobas e** 402 analyzer; y) with the Elecsys CA 15-3 II, REF 07027001190 (**cobas e** 801 analyzer; x) gave the following correlations (U/mL):

Number of samples measured: 138

Passing/Bablok[9]	Linear regression
y = 1.07x - 0.190	y = 1.06x + 0.043
τ = 0.960	r = 0.994

The sample concentrations were between 1.91 and 273 U/mL.

Analytical specificity

The Elecsys CA 15-3 II assay is based on the monoclonal 115D8 and DF3 antibodies which are only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of test procedures using these antibodies cannot be assumed for test methods using other antibodies.

Clinical performance in follow-up

Patients diagnosed with breast cancer were examined in a retrospective study (at least 4 samples/patient during follow-up study) and classified as recurrence [yes/no] after no evidence of breast cancer or response to treatment [yes/no] after breast cancer metastasis based on the clinical information (medical imaging and other clinical investigations). The CA 15-3 concentrations were measured in parallel. The ROC (receiver-operating characteristics) analysis of relative CA15-3 change to determine breast cancer recurrence/ therapy response in metastasized breast cancer was done to show clinical accuracy at various cut-offs and to summarize the cutoff-independent clinical performance in a ROC plot and the related AUC (area under the curve).

Early detection of recurrence

Forty (40) patients treated for stage II or III breast cancer were followed for up to 1351 days (median 105 days). A total of 172 samples (median 4 samples per patient) were assessed for recurrence of disease over the follow-up period. Recurrence was defined as the presence of clinical symptoms in women with no evidence of disease at the beginning of the follow-up period. Eighteen (18) patients experienced recurrence of disease.

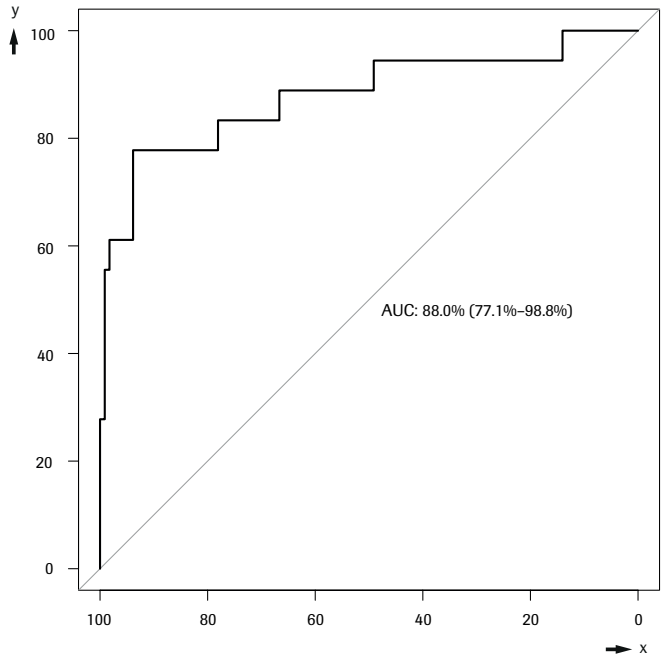
2 x 2 table for early detection of recurrence:

	recurrence	
CA15-3 increase > 25%	no	yes
no	93	4
yes	21	14

The corresponding results for positive predictive value (PPV) and negative predictive value (NPV) with the 95 % confidence interval for a cutoff of 25 % CA 15-3 increase as derived from the table are:

Positive predictive value: 40 % (24-58 %)

Negative predictive value: 90 % (90-99 %)



x = Specificity (%); y = Sensitivity (%)

Figure 1: ROC curve: breast cancer recurrence by relative change CA 15-3 to baseline

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The area under the curve (AUC) was 0.8796 (95 % CI: 0.7709-0.9884)

Monitoring response to therapy

Fifteen (15) patients with metastatic breast cancer underwent treatment and response to therapy was assessed by clinical criteria. A total of 72 assessments (median 4 assessments per patient) were made. Fourteen (14) patients had a response to therapy.

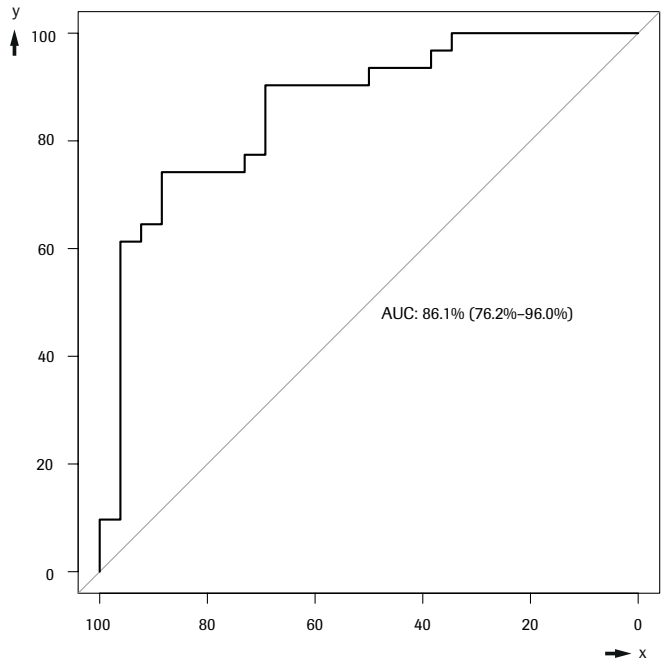
2 x 2 table for response to therapy

	response	
CA15-3 decrease > 25%	no	yes
no	25	19
yes	1	12

The corresponding results for positive predictive value (PPV) and negative predictive value (NPV) with the 95 % confidence interval as derived from the table are:

Positive predictive value: 92 % (64-100 %)

Negative predictive value: 57 % (41-72 %)



x = Specificity (%); y = Sensitivity (%)

Figure 2: ROC curve: breast cancer response to therapy by relative change CA 15-3 to baseline

The area under the curve (AUC) was 0.8610 (95% CI: 0.7623-0.9598).

References

1 Duffy MJ. CA 15-3 and related mucins as circulating markers in breast cancer. Ann Clin Biochem,1999;36:579-586.

2 Hilkens J, Buijs F, Hilgers J, et al. Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland. Prot Biol Fluids 1982;29:813-816.

3 Hilkens J, Buijs F, Hilgers J, et al. Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. Int J Cancer 1984;34:197-206.

4 Kufe D, Inghirami G, Abe M, et al. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumor. Hybridoma 1984;(3):223-232.

5 Sekine H, Ohno T, Kufe DW. Purification and characterization of a high molecular weight glycoprotein detectable in human milk and breast carcinomas. J Immunol 1985;135(5):3610-3615.

6 Ponnusamy MP, Seshacharyulu P, Lakshmanan I, et al. Emerging role of mucins in epithelial to mesenchymal transition. Curr cancer drug targets 2013;13(9):945-56.

7 Duffy MJ, Walsh S, McDermott EW, et al. Chapter One - Biomarkers in Breast Cancer: Where Are We and Where Are We Going?, In: Gregory S. Makowski, Editor(s), Advances in Clinical Chemistry, Elsevier, 2015;71: 1-23, ISSN 0065-2423, ISBN 9780128022566.

8 Cardoso F, et al. ESMO Guidelines for advanced breast cancer. Annals of Oncology,2018;29: 1634-1657.

9 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

FUJIREBIO Diagnostics, Inc.

CA 15-3 is a registered trademark of Fujirebio Diagnostics, Inc.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

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REF		Σ	SYSTEM
07027192190	07027192500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
DHEAS	10068

Intended use

Immunoassay for the in vitro quantitative determination of dehydroepiandrosterone sulfate (DHEA-S) in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Dehydroepiandrosterone sulfate (DHEA-S) measurements, performed with this assay, in human serum and plasma are used as an aid in diagnosis and differential diagnosis of androgens related endocrine function such as hyperandrogenism, adrenal tumors and premature adrenarche.

DHEA-S is a steroid hormone synthetized in the zona reticularis of the adrenal glands in response to adrenocorticotropic hormone (ACTH). Like other steroids, DHEA-S is synthesized from cholesterol. DHEA-S is hormonally inert, but it can be converted to other more potent androgens or estrogens. Therefore DHEA-S can be considered a prohormone.

During fetal development DHEA-S is produced in the adrenal gland and the level declines rapidly during the first year of life. The production of DHEA-S resumes again during adrenarche, increases during puberty and reaches the peak values between 20 and 30 years of age. Thereafter DHEA-S levels steadily decline. In males the adrenal glands account for only a small amount of the total androgen production, while in females of reproductive age the adrenal contribution to androgen production is more pronounced.[1]

Measurement of DHEA-S may be useful in the diagnostic work-up of female subjects presenting with signs and symptoms of hyperandrogenism. Hyperandrogenism is usually caused by excessive androgen production by the ovaries, the adrenal glands, or both.[2]

The most common disease associated with hyperandrogenism is polycystic ovary syndrome (PCOS).[3] Other diseases where the measurement of DHEA-S may be useful are nonclassic congenital adrenal hyperplasia[4], androgen-secreting adrenal tumors[5], Cushing syndrome and hyperprolactinemia.[1,2]

A high DHEA-S level may indicate an adrenal factor in androgen production[6], and if substantially elevated, the presence of an adrenocortical neoplasm.[7] DHEA-S measurements are also useful in the determination of premature adrenarche in children.[8]

The Elecsys DHEA-S assay makes use of a competition test principle using a polyclonal antibody (rabbit) specifically directed against DHEA-S. Endogenous DHEA-S in the sample competes with added DHEA-S derivative labeled with a ruthenium complex[a)] for the binding sites on the biotinylated antibody.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (9 µL) with a DHEA-S-specific biotinylated antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and a DHEA-S derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

Reagents - working solutions

The **cobas e** pack is labeled as DHEAS.

M Streptavidin-coated microparticles, 1 bottle, 6.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-DHEA-S-Ab~biotin, 1 bottle, 9.9 mL:
Biotinylated polyclonal anti-DHEA-S antibody (rabbit) 450 ng/mL; phosphate buffer 100 mmol/L, pH 6.8; preservative.

R2 DHEA-S~$Ru(bpy)_3^{2+}$, 1 bottle, 9.9 mL:
DHEA-S derivative (synthetic) labeled with ruthenium complex 0.32 ng/mL; phosphate buffer 100 mmol/L, pH 6.8; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

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Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 12 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03000095122, DHEA-S CalSet, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against gravimetrically produced master calibrators consisting of exactly defined DHEA-S concentrations in depleted human serum matrix.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

Use PreciControl Universal or other suitable controls for routine quality control procedures.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in µmol/L, µg/dL or µg/mL).

Conversion factors:

µmol/L x 36.846 = µg/dL

µg/dL x 0.02714 = µmol/L

µg/dL x 0.01 = µg/mL

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 222 µmol/L or ≤ 13 mg/dL
Hemoglobin	≤ 0.35 mmol/L or ≤ 0.56 g/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70 ng/mL
Rheumatoid factors	≤ 80 IU/mL

Criterion: For concentrations of 0.2-50 µg/dL the deviation is ≤ ± 5 µg/dL. For concentrations > 50 µg/dL the deviation is ≤ ± 10 %.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

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For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.005-27.1 µmol/L or 0.2-1000 µg/dL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.005 µmol/L or < 0.2 µg/dL. Values above the measuring range are reported as > 27.1 µmol/L or > 1000 µg/dL (or up to 135.7 µmol/L or 5000 µg/dL for 5-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.003 µmol/L (0.1 µg/dL)

Limit of Detection = 0.005 µmol/L (0.2 µg/dL)

Limit of Quantitation = 0.081 µmol/L (3 µg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with DHEA-S concentrations above the measuring range can be diluted using human samples with a low analyte concentration. The recommended dilution is 1:5. The concentration of the diluted sample must be > 1.22 µmol/L (> 45 µg/dL).

If the endogenous DHEA-S concentration is negligible, multiply the result by the dilution factor or calculate using the following equation:

C = c + 4 (c - D)

C = true DHEA-S concentration of the sample

c = measured DHEA-S concentration

D = DHEA-S concentration in the diluent (human sample)

Expected values

Extended studies with the Elecsys DHEA-S assay conducted in two clinical centers in Germany covering a total of 519 samples from female individuals, a total of 489 samples from male individuals and a total of 269 samples from children gave the following values for the age groups listed below (study protocols No.: C00P032 and C01P005 - status 05/01 to 11/01):

Age (years)	N	50th percentile		5-95th percentile	
		µmol/L	µg/dL	µmol/L	µg/dL
Females:					
10-14	73	3.34	123	0.92-7.60	33.9-280
15-19	55	4.26	157	1.77-9.99	65.1-368
20-24	36	6.46	238	4.02-11.0	148-407
25-34	64	4.96	183	2.68-9.23	98.8-340
35-44*	85	4.38	161	1.65-9.15	60.9-337
45-54*	89	3.28	121	0.96-6.95	35.4-256
55-64	59	2.08	76.7	0.51-5.56	18.9-205
65-74	29	1.75	64.4	0.26-6.68	9.40-246
≥ 75	29	1.65	60.9	0.33-4.18	12.0-154
Males:					
10-14	74	2.74	101	0.66-6.70	24.4-247
15-19	67	7.57	279	1.91-13.4	70.2-492
20-24	28	9.58	353	5.73-13.4	211-492
25-34	60	7.68	283	4.34-12.2	160-449
35-44	70	6.00	221	2.41-11.6	88.9-427
45-54	45	5.94	219	1.20-8.98	44.3-331
55-64	69	3.75	138	1.40-8.01	51.7-295
65-74	55	2.45	90.2	0.91-6.76	33.6-249
≥ 75	21	1.53	56.2	0.44-3.34	16.2-123
Children:					
< 1 week	37	7.60	280	2.93-16.5	108-607
1-4 weeks	25	3.91	144	0.86-11.7	31.6-431
1-12 months	69	0.59	21.6	0.09-3.35	3.4-124
1-4 years	59	0.14	5.0	0.01-0.53	0.47-19.4
5-9 years	79	0.63	23.1	0.08-2.31	2.8-85.2

* Effects of the menopause on the results obtained for the women of the corresponding age groups were tested and found to be negligible.

DHEA-S values of newborns are strongly influenced by maternal hormonal exchange via placenta.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	µmol/L	µg/dL	µmol/L	µg/dL	%	µmol/L	µg/dL	%
HS[b)] 1	0.012	0.436	0.027	0.160	36.6	0.005	0.174	39.9
HS 2	0.131	4.82	0.015	0.539	11.2	0.018	0.667	13.8
HS 3	8.47	312	0.315	11.6	3.7	0.413	15.2	4.9
HS 4	19.0	699	0.662	24.4	3.5	0.801	29.5	4.2
HS 5	25.5	939	0.912	33.6	3.6	1.29	47.7	5.1
PC[c)] Universal1	5.56	205	0.190	7.01	3.4	0.225	8.29	4.0
PC Universal2	15.4	492	0.426	15.7	3.2	0.619	22.8	4.6

b) HS = human serum

c) PC = PreciControl

Method comparison

A comparison of the Elecsys DHEA-S assay, REF 07027192190 (**cobas e** 801 analyzer; y) with the Elecsys DHEA-S assay, REF 03000087122 (**cobas e** 601 analyzer; x) gave the following correlations (µg/dL):

Number of samples measured: 148

Elecsys DHEA-S

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Passing/Bablok[9]	Linear regression
y = 0.986x - 0.715	y = 0.998x - 3.26
τ = 0.971	r = 0.997

The sample concentrations were between 0.252 and 980 µg/dL.

A comparison of the Elecsys DHEA-S assay, REF 07027192190 (**cobas e** 402 analyzer; y) with the Elecsys DHEA-S assay, REF 07027192190 (**cobas e** 801 analyzer; x) gave the following correlations (µg/dL):

Number of samples measured: 202

Passing/Bablok[9]	Linear regression
y = 1.016x - 1.37	y = 1.006x +1.90
τ = 0.980	r = 0.998

The sample concentrations were between 2.19 and 944 µg/dL.

Analytical specificity

For the Elecsys DHEA-S assay, the following cross-reactivities were found:

Substance	Cross-reactivity %	Additive concentration µg/dL
Androstenedione	10.8	1000
DHEA	8.90	1000
Androsterone	2.10	2000
Testosterone	2.55	2000
Aldosterone	0.320	5000
Androsterone-sulfate	1.10	5000
DHEA-glucuronide	2.08	5000
Estradiol	n. d.[d)]	5000
Estriol	n. d.	5000
Estrone	0.740	5000
Estrone-3-sulfate	0.500	5000
Progesterone	1.32	5000
5-α-Dihydrotestosterone	1.12	5000
19-Hydroxyandrostendione	1.66	5000
Cortisol	0.060	10000

d) n. d. = not detectable

References

1 Bertholf RLB, Cooper M, Winter WE. Adrenal Cortex. Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, ch. 56, p. 805-805.e37.

2 Goodman NF, Bledsoe MB, Cobin RH, et al. American Association of Clinical Endocrinologists Hyperandrogenic Disorders Task Force. American Association of Clinical Endocrinologists medical guidelines for the clinical practice for the diagnosis and treatment of hyperandrogenic disorders. Endocr Pract. 2001 Mar-Apr;7(2):120-34.

3 Teede HJ, Tay CT, Laven J, et al. International PCOS Network. Recommendations from the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome†. Hum Reprod. 2023 Sep 5;38(9):1655-1679.

4 Speiser PW, Arlt W, Auchus RJ, et al. Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2018 Nov 1;103(11):4043-4088.

5 Fassnacht M, Dekkers OM, Else T, et al. European Society of Endocrinology Clinical Practice Guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol. 2018 Oct 1;179(4):G1-G46.

6 Goodarzi MO, Carmina E, Azziz R. DHEA, DHEAS and PCOS. J Steroid Biochem Mol Biol. 2015 Jan;145:213-25.

7 Pugeat M, Déchaud H, Raverot V, et al. Recommendations for investigation of hyperandrogenism. Ann Endocrinol (Paris). 2010 Feb;71(1):2-7.

8 Rosenfield RL. Normal and Premature Adrenarche. Endocr Rev. 2021 Nov 16;42(6):783-814.

9 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

Elecsys Estradiol III

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REF		Σ	SYSTEM
07027249190* 07027249214*	07027249500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
E2 3	10100

Intended use

Immunoassay for the in vitro quantitative determination of estradiol in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Estradiol measurements, performed with this assay, in human serum and plasma are used as an aid in diagnosing disorders of the hypothalamus-pituitary-gonad axis, assessment of ovarian function and monitoring of fertility therapy.

Estrogens are responsible for the development of the secondary female sex characteristics.[1] Estradiol is a C18 steroid hormone of the estrogen family[2] and constitutes the major gonadal steroid involved in the pubertal growth spurt in females.[3] Like other steroid hormones, estradiol is derived from cholesterol.[4]

Estrogens are secreted primarily in healthy women by the ovarian follicles and the corpus luteum and during pregnancy by the placenta. The adrenal glands and testes (in men) are also believed to secrete minute quantities of estrogens.[4]

Levels of estradiol in premenopausal women are highly variable throughout the menstrual cycle.

Estradiol concentrations decrease abruptly after ovulation but increase again as the corpus luteum is formed during the luteal phase. Together with the progesterone produced by the corpus luteum, estradiol exerts a negative effect on the hypothalamus and anterior lobe of the pituitary gland and LH and FSH secretion is suppressed again during the luteal phase. The decrease in negative feedback from estradiol on the anterior lobe of the pituitary gland triggers the FSH surge, which begins the development of an ovarian follicle for the next cycle.[4]

The major fraction of estradiol (about 97 %) circulates in blood bound with high affinity to sex-hormone binding globulin (SHBG) and with lower affinity to albumin. The unbound fraction (between 1–3 %) is considered to be the biologically active fraction.[2] Estradiol concentrations can span multiple orders of magnitude among different age groups, between males and females, and under different conditions (e.g. fertility treatments, pregnancy, use of aromatase inhibitors).[5]

The determination of estradiol is utilized clinically in the diagnosis of disorders of the hypothalamus-pituitary-gonad axis such as gynecomastia,[6] in case of estrogen-producing ovarian and testicular tumors,[7,8] in the context of fertility disorders such as polycystic ovary syndrome[9] and within the framework of in vitro fertilization (IVF).[10,11]

The Elecsys Estradiol III assay employs a competitive test principle using two monoclonal antibodies specifically directed against 17β-estradiol. Endogenous estradiol released from the sample by mesterolone competes with the added estradiol derivative labeled with a ruthenium complex[a)] for the binding sites on the biotinylated antibody.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (15 µL) with two estradiol-specific biotinylated antibodies, immunocomplexes are formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and an estradiol derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

Reagents - working solutions

The **cobas e** pack is labeled as E2 3.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-estradiol-Ab~biotin, 1 bottle, 19.7 mL:
Two biotinylated monoclonal anti-estradiol antibodies (rabbit) 2.5 ng/mL and 4.5 ng/mL; mesterolone 130 ng/mL; MES[b)] buffer 50 mmol/L, pH 6.0; preservative.

R2 Estradiol-peptide~$Ru(bpy)_3^{2+}$, 1 bottle, 18.8 mL:
Estradiol derivative, labeled with ruthenium complex 4.5 ng/mL; MES buffer 50 mmol/L, pH 6.0; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

Elecsys Estradiol III

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P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Li-heparin plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 10 pg/mL + coefficient of correlation ≥ 0.95.

Stable for 24 hours at 20-25 °C, 2 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 06656048190, Estradiol III CalSet, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- REF 07299010190, Diluent MultiAssay, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against CRM 6004a via ID-GC/MS (isotope dilution-gas chromatography/mass spectrometry).[12]

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L, pg/mL, ng/L or nmol/L).

Conversion factors: pmol/L x 0.272 = pg/mL (ng/L)

pg/mL x 3.67 = pmol/L

pg/mL x 0.00367 = nmol/L

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1000 mg/dL
Biotin	≤ 147 nmol/L or ≤ 36 ng/mL

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Compound	Concentration tested
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 70 g/L
IgA	≤ 0.4 g/dL
IgM	≤ 10 g/L
Albumin	≤ 5 g/dL

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

Erroneous test results may be obtained from samples taken from patients who have been exposed to vaccines containing rabbit serum or when keeping rabbits as pet animals.

Due to the risk of cross reactivity, this assay should not be used when monitoring Estradiol levels in patients being treated with Fulvestrant.

Steroid drugs may interfere with this test.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

18.4-11010 pmol/L (5-3000 pg/mL) (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 18.4 pmol/L or < 5 pg/mL. Values above the measuring range are reported as > 11010 pmol/L or > 3000 pg/mL (or up to 110100 pmol/L or 30000 pg/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 11.0 pmol/L (3 pg/mL)

Limit of Detection = 18.4 pmol/L (5 pg/mL)

Limit of Quantitation = 91.8 pmol/L (25 pg/mL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 30 %.

Dilution

Samples with estradiol concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the analyzer or manually). The concentration of the diluted sample must be ≥ 881 pmol/L (≥ 240 pg/mL).

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

The endogenous analyte concentration of the diluent (< 220 pmol/L or < 60.0 pg/mL) is not taken into account for dilutions above the measuring range.

Expected values

The expected ranges were determined by testing specimens drawn from 150 apparently healthy males, 142 apparently healthy, post-menopausal women over the age of 50, and from 412 apparently healthy pregnant women between the ages of 18 and 50 (136 in the first trimester, 140 in the second trimester, and 136 in the third trimester). The expected range for healthy women was determined by collecting blood at multiple time points of one menstrual cycle from 85 apparently healthy subjects with a natural menstrual cycle that were not taking any hormonal contraceptives. A menstrual cycle was defined as the phase between two subsequent menstrual bleedings. Cycle length (29 days) and day of ovulation (day 15) were standardized to account for variation in cycle length within the study population and to enable determination of expected values for further sub-phases. Only ovulatory menstrual cycles were used for value analysis. The following ranges were obtained:

Test subjects	N	2.5th percentile pmol/L (90 % CI*)	Median pmol/L (90 % CI)	97.5th percentile pmol/L (90 % CI)
Healthy men	150	41.4 (22.4-49.0)	90.9 (84.9-97.7)	159 (151-337)
Healthy postmenopausal women				
• Postmenopause	142	< 18.4 (< 18.4-< 18.4)	< 18.4 (< 18.4-19.2)	505 (189-1151)
Healthy pregnant women				
• 1st trimester	136	563 (467-636)	3133 (2703-4004)	11902 (9891-15271)
• 2nd trimester	140	5729 (4173-7457)	28402 (24207-32090)	78098 (69143-92227)
• 3rd trimester	136	31287 (27151-34175)	64684 (62353-68189)	> 110100 (107164-> 110100)

* CI = confidence interval

Healthy women Cycle Phase	N **	5th percentile pmol/L (90 % CI)	Median pmol/L (90 % CI)	95th percentile pmol/L (90 % CI)
Follicular	85	114 (19.1-135)	198 (188-208)	332 (322-637)
Ovulation	81	222 (98.5-283)	757 (667-944)	1959 (1598-3338)
Luteal	85	222 (159-280)	412 (390-488)	854 (760-1334)

**N = number of patients contributing to the data in this menstrual cycle phase (not number of samples); differences in N per phase are due to cycle standardization procedure

Healthy women Cycle Sub-Phase	N	5th percentile pmol/L (90 % CI)	Median pmol/L (90 % CI)	95th percentile pmol/L (90 % CI)
Early follicular	78	75.5 (< 18.4-78.5)	125 (120-135)	231 (192-283)
Intermediate follicular	83	95.6 (19.1-114)	172 (159-180)	294 (262-695)
Late follicular	84	182 (84-215)	464 (424-519)	858 (711-1337)
Ovulation	79	222 (98.5-283)	817 (724-974)	2212 (1598-3338)
Early luteal	85	188 (163-218)	390 (330-412)	658 (608-1394)

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Healthy women Cycle Sub-Phase	N	5th percentile pmol/L (90 % CI)	Median pmol/L (90 % CI)	95th percentile pmol/L (90 % CI)
Intermediate luteal	81	244 (157-334)	505 (445-568)	1123 (942-1538)
Late luteal	84	111 (74.4-163)	396 (373-422)	815 (703-908)

Test subjects	N	2.5th percentile pg/mL (90 % CI)	Median pg/mL (90 % CI)	97.5th percentile pg/mL (90 % CI)
Healthy men	150	11.3 (6.1-13.4)	24.8 (23.1-26.6)	43.2 (41.0-91.9)
Healthy postmenopausal women				
• Postmenopause	142	< 5 (< 5-< 5)	<5 (< 5-5.24)	138 (51.6-314)
Healthy pregnant women				
• 1st trimester	136	154 (127-173)	854 (737-1091)	3243 (2695-4161)
• 2nd trimester	140	1561 (1137-2032)	7739 (6596-8744)	21280 (18840-25130)
• 3rd trimester	136	8525 (7398-9312)	17625 (16990-18580)	> 30000 (29200-> 30000)

Healthy women Cycle Phase	N	5th percentile pg/mL (90 % CI)	Median pg/mL (90 % CI)	95th percentile pg/mL (90 % CI)
Follicular	85	30.9 (5.21-36.7)	53.9 (51.1-56.6)	90.4 (87.7-173)
Ovulation	81	60.4 (26.8-77)	206 (181-257)	533 (435-908)
Luteal	85	60.4 (43.2-76)	112 (106-133)	232 (207-363)

Healthy women Cycle Sub-Phase	N	5th percentile pg/mL (90 % CI)	Median pg/mL (90 % CI)	95th percentile pg/mL (90 % CI)
Early follicular	78	20.5 (< 5-21.4)	34 (32.6-36.7)	62.8 (52.1-77)
Intermediate follicular	83	26 (5.21-31)	46.9 (43.2-49)	79.8 (71.4-189)
Late follicular	84	49.5 (22.8-58.5)	126 (115-141)	233 (193-364)
Ovulation	79	60.4 (26.8-77)	222 (197-265)	602 (435-908)
Early luteal	85	51.1 (44.3-59.2)	106 (89.8-112)	179 (166-379)
Intermediate luteal	81	66.5 (42.7-90.7)	137 (121-155)	305 (256-418)
Late luteal	84	30.2 (20.2-44.3)	108 (101-115)	222 (191-247)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	68.6	5.73	8.4	8.44	12.3
Human serum 2	5832	67.5	1.2	111	1.9
Human serum 3	701	9.84	1.4	12.9	1.8
Human serum 4	1718	20.8	1.2	34.7	2.0
Human serum 5	10129	244	2.4	276	2.7
PC[c)] Universal 1	307	6.24	2.0	7.96	2.6
PC Universal 2	1486	23.3	1.6	25.4	1.7

c) PC = PreciControl

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	18.7	1.56	8.4	2.30	12.3
Human serum 2	1589	18.4	1.2	30.3	1.9
Human serum 3	191	2.68	1.4	3.51	1.8
Human serum 4	468	5.68	1.2	9.45	2.0
Human serum 5	2760	66.5	2.4	75.3	2.7
PC Universal 1	83.6	1.70	2.0	2.17	2.6
PC Universal 2	405	6.35	1.6	6.91	1.7

Method comparison

a) A comparison of the Elecsys Estradiol III assay, REF 07027249190 (**cobas e** 801 analyzer; y) with the Elecsys Estradiol III assay, REF 06656021190 (**cobas e** 601 analyzer; x) gave the following correlations (pg/mL):

Number of samples measured: 130

Passing/Bablok[13]	Linear regression
y = 1.008x + 0.381	y = 0.998x + 1.53
τ = 0.980	r = 1.000

The sample concentrations were between 7.26 and 2909 pg/mL.

b) A comparison of the Elecsys Estradiol III assay, REF 07027249190 (**cobas e** 402 analyzer; y) with the Elecsys Estradiol III assay, REF 07027249190 (**cobas e** 801 analyzer; x) gave the following correlations (pg/mL):

Number of serum samples measured: 190

Passing/Bablok[13]	Linear regression
y = 1.03x + 2.09	y = 1.03x + 1.83
τ = 0.988	r = 1.00

The sample concentrations were between 10.8 and 2861 pg/mL.

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Analytical specificity

For the Elecsys Estradiol III assay, the following cross-reactivities were found:

Substance	Cross-reactivity %	Additive concentration ng/mL
6-α-Hydroxy-Estradiol	102	1
4-Hydroxyestradiol	3.073	10
Aldosterone	n. d.[d)]	100
Androstenedione	0.005	100
Equiline	0.032	100
Estriol	0.325	100
Estrone	0.761	100
Estrone-3β-glucuronide	0.001	100
Estrone-3-sulfate	0.001	100
Ethisterone	0.006	100
Norethindrone acetate	n. d.	100
Pregnenolone	n. d.	100
Progesterone	n. d.	100
2-Methoxyestradiol	0.028	100
17β-Estradiol-3,17-sulfate	n. d.	100
17β-Estradiol-3-β-D-glucuronide	0.007	100
17β-Estradiol-17-β-D-glucuronide	n. d.	100
17β-Estradiol-3-glucuronide-17-sulfate	0.002	100
17β-Estradiol-3-sulfate-17-glucuronide	0.006	100
17β-Estradiol-3-sulfate	0.014	100
17β-Estradiol-17-valerate	0.059	100
17β-Estradiol-17-sulfate	0.016	100
2-Hydroxyestradiol	0.053	100
17-Hydroxyprogesterone	n. d.	100
17-α-Ethinylestradiol	0.279	200
Cortisol	0.004	200
Cortisone	0.002	200
Tamoxifen	n. d.	200
Chlomiphene	n. d.	250
Prednisolone	n. d.	1000
Danazol	n. d.	10000
DHEA-S	n. d.	10000
Mesterolone	n. d.	10000
Testosterone	n. d.	10000
5-α-Dihydrotestosterone (DHT)	n. d.	10000
5-Androstene-3β-,17β-diol	n. d.	10000

d) n. d. = not detectable

References

1 Hall JE, Hall ME. Female Physiology Before Pregnancy and Female Hormones. In: Hall JE, Hall ME, editors. Guyton and Hall Textbook of Medical Physiology, Elsevier,14th edition, 2021, chapter 82, p.1027-44.

2 Ratcliffe WA, Carter GD, Dowsett M, et al. Oestradiol assays: applications and guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem 1988 Sep;25 (Pt 5):466-83. doi: 10.1177/000456328802500502.

3 Frank GR. Role of estrogen and androgen in pubertal skeletal physiology. Med Pediatr Oncol 2003 Sep;41(3):217-21. doi: 10.1002/mpo.10340.

4 Nerenz RD, Boh B. Reproductive endocrinology and related disorders. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 58, p. 846-884.e11.

5 Smy L, Straseski JA. Measuring estrogens in women, men, and children: Recent advances 2012-2017. Clin Biochem 2018 Dec;62:11-23. doi: 10.1016/j.clinbiochem.2018.05.014.

6 Kanakis GA, Nordkap L, Bang AK, et al. EAA clinical practice guidelines-gynecomastia evaluation and management. Andrology 2019 Nov;7(6):778-793. doi: 10.1111/andr.12636.

7 Roth LM, Billings SD. Hormonally Functional Ovarian Neoplasms. Endocr Pathol 2000 Spring;11(1):1-17.

8 Braunstein GD. Gynecomastia. N Engl J Med 1993 Feb18;328(7):490-5. doi: 10.1056/NEJM199302183280708.

9 Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2013 Dec;98(12):4565-92. doi: 10.1210/jc.2013-2350.

10 Zhang XJ, Liu SY, Fu W, et al. The association of serum estradiol level with outcomes of clomiphene citrate/human menopausal gonadotropin ovarian stimulation for in vitro fertilization and embryo transfer. Reprod Biol Endocrinol 2015 Oct 6;13:114. doi: 10.1186/s12958-015-0109-x.

11 Rosner W, Hankinson SE, Sluss PM, et al. Challenges to the measurement of estradiol: an endocrine society position statement. J Clin Endocrinol Metab 2013 Apr;98(4):1376-87. doi: 10.1210/jc.2012-3780.

12 Thienpont LM, Verhseghe PG, Van Brussel KA, et al. Estradiol-17-beta quantified in serum by isotope dilution-gas chromatography-mass spectrometry. Clin Chem 1988(34);10:2066-2069.

13 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

Elecsys Estradiol III

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CE 0123

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Roche

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REF		Σ	SYSTEM
08324174190	08324174500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)	Application
FOL 3	10168	Folate serum/plasma
RBC 2	10169	Folate RBC application

Intended use

Binding assay for the in vitro quantitative determination of folate in human serum, plasma and erythrocytes (red blood cells, RBC). Folate measurements, performed with the Elecsys Folate III assay, are used as an aid in diagnosis and monitoring of folate imbalance.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Folate deficiency may be due to several clinical conditions such as decreased nutritional intake, poor absorption of ingested folate in the intestine, increased demand of folate (during physical activity or pregnancy), liver diseases, impaired folate metabolism due to genetic defects or due to drug interactions. Folate measurements are also used to aid in diagnosis of megaloblastic (macrocytic) anemia.

Folate belongs to the family of B-group vitamins composed of an aromatic pteridine ring linked through a methylene group to p-aminobenzoic acid and a glutamate residue. Folate (folic acid) is vital for normal cellular functions and plays an essential role in nucleic acid synthesis, methionine regeneration, shuttling and redox reactions of one-carbon-units required for normal metabolism and regulation.[1,2]

The folate metabolism can be exemplified as a cycle, where folate facilitates the transfer of one-carbon-units from one molecule to another required in various biochemical reactions: for example, tetrahydrofolate (THF) accepts a single carbon unit from serine, which is reduced in a number of steps to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF gives its methyl group to homocysteine, which is - with involvement of methionine synthase and vitamin B12 - enzymatically converted to methionine. The resulting THF starts again the cycle of methyl group synthesis. From methionine, the methyl groups are transferred to S-adenosylmethionine (SAM).[3] SAM serves as a methyl group donor in several methylation reactions, like DNA, RNA and protein methylation.[1]

The methionine cycle is highly sensitive to folate deficiency: with a low folate status, the ability of the cell to re-methylate homocysteine is impaired and this results in increased homocysteine concentrations in plasma.[2]

Folate also plays an essential role in the synthesis of purine and pyrimidine precursors of nucleic acids. Altered distribution of methyl groups and impaired DNA synthesis play an essential role in the development of cancers. Abnormal folate status has also been linked with the development of diseases like cardiovascular diseases, neural tube defects, cleft lip and palate, late pregnancy complications, neurodegenerative and psychiatric disorders.[1,2]

Folate belongs to the group of essential vitamins, i.e. it cannot be synthesized by the human organism and therefore must be absorbed from diet. Primary sources of folates are green and leafy vegetables, sprouts, fruits, brewer's yeast and liver.[1,2]

In children, the demand of folate is particularly high during the period of rapid growth.[4] The normal infant requirement is 25-35 µg/day, and weight-based requirements are higher in children compared to adults due to the increased needs of folate to support growth.

In children, the normal range of RBC folate is 150-600 ng/mL.[5] and the RBC-folate cutoff value of < 151 ng/mL (< 340 nmol/L) indicates folate deficiency in all age groups, including children.[6,7]

Serum folate concentrations are higher in small children, and the level decreases with age in both sexes.[8,9] The cutoff recommended by WHO to be used to determine folate deficiency is < 4 ng/mL (< 10 nmol/L) in serum, the same cutoff can be applied to all ages.[6]

During pregnancy, the mother undergoes both anatomical and physiological changes to enable the fetus to develop and grow. These changes include a progressive increase in plasma volume, but the expansion of plasma volume is greater than the increase in red blood cell mass, which leads to a fall in the hemoglobin concentration, haematocrit and RBC count.[10] These changes may influence the folate concentrations in pregnant women.

Folate is essential for fetal development, and guidelines recommend women that are pregnant or are planning to become pregnant to take folic acid supplements at a concentration of 400 µg/day to prevent fetal malformations such as neural tube defects, but also other pregnancy complications such as preeclampsia.[11,12,13] If not supplemented during pregnancy and lactation, folate levels decrease in both plasma and RBC.[14] Folic acid supplements of 400 µg/day are to ensure that the women achieve an RBC folate cutoff of 906 nmol/L, which is the value associated with maximal reduction of the risk of neural tube defect.[15,16] By examining the association between the folate concentrations in plasma and in RBC, an estimated plasma-folate insufficiency cutoff of 25.5 nmol/L was found to correspond to the RBC-folate insufficiency cutoff of 906 nmol/L.[17]

A clinical manifestation of both folate and vitamin B12 deficiency is the so called megaloblastic (macrocytic) anemia: due to the affected DNA synthesis and cell maturation, especially involving the cells of erythropoiesis, the total count of erythrocytes is significantly reduced. The hemoglobin synthesis capacity however is normal, which leads to abnormally large erythrocyte precursors ("macrocytes" or "megaloblasts"), which have an elevated hemoglobin content ("hyperchromic anemia").[3,18]

Because vitamin B12 and folate are closely interrelated in the cellular one-carbon-unit metabolism, and also hematologic and clinical consequences of the two vitamin-deficiency states might be similar, it is advisable to determine both parameters simultaneously in patients with the relevant symptoms of vitamin-deficiency.[3,18]

1. Folate serum/plasma application

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating 15 µL of sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as FOL 3.

PT1 Pretreatment reagent 1, 1 bottle, 7.3 mL:
Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT2 Pretreatment reagent 2, 1 bottle, 7.3 mL:
Sodium hydroxide 25 g/L.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

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R1 Folate-binding protein~Ru(bpy)$_3^{2+}$, 1 bottle, 16.7 mL:
Ruthenium-labeled folate-binding protein 75 µg/L; human serum albumin (stabilizer); phosphate buffer 70 mmol/L, pH 5.5; preservative.

R2 Folate~biotin, 1 bottle, 13.9 mL:
Biotinylated folate 17 µg/L; human serum albumin (stabilizer); bis-tris propane buffer 100 mmol/L, pH 9.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

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

Response:

P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

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

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
Immediately call a POISON CENTER/ doctor.

P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[19,20]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The Elecsys Folate III kit can be used for both the folate serum/plasma application and the folate RBC application.

Both applications use the same reagents.

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin plasma.

Li-heparin plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1, coefficient of correlation ≥ 0.95.

Stable for 2 hours at 20-25 °C, 48 hours at 2-8 °C, 28 days at -20 °C (± 5 °C). Freeze only once. Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Note: Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay. Samples for folate determinations should be collected from fasting persons.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 08324247190, CalSet Folate, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M

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- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This application has been standardized against the WHO International Standard NIBSC Code 03/178.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Varia.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L or ng/mL).

Conversion factors: nmol/L x 0.44 = ng/mL

ng/mL x 2.27 = nmol/L

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 496 µmol/L or ≤ 29 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1000 IU/mL
IgG	≤ 1.6 g/dL
IgA	≤ 0.4 g/dL
IgM	≤ 1 g/dL

Criterion: For concentrations of 0.6-4 ng/mL the deviation is ≤ 0.4 ng/mL. For concentrations > 4 ng/mL the deviation is ≤ 10 %.

Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay.

Samples with extremely high total protein concentrations (hyperproteinemia) are not suitable for use in this assay. Hyperproteinemia may be caused by, but not limited to, the following conditions: Lymphoma,[21,22] bone marrow disorders such as multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Waldenström macroglobulinemia, plasmocytoma,[21,22,23,24,25,26,27] amyloidosis.[27,28] Respective samples may lead to the formation of protein gel in the assay cup, which may cause a run abort. The critical total protein concentration is dependent upon the individual sample composition.

Pharmaceutical substances

In vitro tests were performed on 15 commonly used pharmaceuticals. No interference with the assay was found. For the common pharmaceuticals cefoxitin and doxycycline no interference was observed for concentrations ≤ 250 mg/L and ≤ 6 mg/L, respectively.

In addition, the following special drug was tested. No interference with the assay was found.

Special drug

Drug	Concentration tested U/mL
Erythropoietin	2000

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.6-20.0 ng/mL or 1.36-45.4 nmol/L (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.6 ng/mL or < 1.36 nmol/L. Values above the measuring range are reported as > 20.0 ng/mL or > 45.4 nmol/L (or up to 40.0 ng/mL or 90.8 nmol/L for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.6 ng/mL (1.36 nmol/L)

Limit of Detection = 1.2 ng/mL (2.72 nmol/L)

Limit of Quantitation = 2.0 ng/mL (4.54 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

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The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

It has been determined using low concentration folate samples.

Dilution

Samples with folate concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the analyzers or manually). The concentration of the diluted sample must be ≥ 8.5 ng/mL or ≥ 19.3 nmol/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Referring to "The American Journal of Clinical Nutrition"[29] serum folate (folic acid) values were found as follows:

Sex	Age	N	Median		2.5th-97.5th percentile	
	years		ng/mL	nmol/L	ng/mL	nmol/L
Both	all	23345	13.0	29.5	4.6-34.8	10.4-78.9
Male	all	11387	12.3	27.9	4.5-32.2	10.2-73.0
Female	all	11958	13.6	30.1	4.8-37.3	10.9-84.5
Both	4-11	3595	17.2	39.0	8.6-37.7	19.5-85.4
Both	12-19	6390	12.1	27.4	5.0-27.2	11.3-61.6
Both	20-59	8689	11.6	26.3	4.4-31.0	10.0-70.2
Both	≥ 60	4671	16.6	37.6	5.6-45.8	12.7-103.8

These values were obtained in the USA during the National Health and Nutrition Examination Survey (NHANES), 1999-2004.

The values shown below were performed on samples from an apparently healthy population, using the Elecsys Folate III assay, REF 07559992190.

The calculation is based on 404 sera (177 men, 227 women). The age range was between 20 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal homocysteine values.

N	Median		2.5th-97.5th percentile	
	ng/mL	nmol/L	ng/mL	nmol/L
404	8.94	20.3	3.89-26.8	8.83-60.8

Please note: These values should only be used as a guideline.

It should be taken into consideration that differences in the expected values may exist with respect to population and dietary status.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Folate deficient sample values

25 samples considered to be deficient[a)] in serum folate concentration were assessed using the Elecsys Folate III assay. All samples were found to be below the 2.5th percentile as given in the table above.

a) Folate deficiency was assessed by measurement of serum folate by 2 commercially available folate assays.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean nmol/L	SD nmol/L	CV %	SD nmol/L	CV %
Human serum 1	4.40	0.336	7.6	0.390	8.9
Human serum 2	8.74	0.447	5.1	0.488	5.6
Human serum 3	10.8	0.384	3.6	0.447	4.1
Human serum 4	21.9	0.804	3.7	0.888	4.1
Human serum 5	41.1	1.31	3.2	1.38	3.4
PreciControl Varia 1	10.2	0.456	4.5	0.511	5.0
PreciControl Varia 2	28.4	0.817	2.9	1.20	4.2

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	1.94	0.148	7.6	0.172	8.9
Human serum 2	3.85	0.197	5.1	0.215	5.6
Human serum 3	4.74	0.169	3.6	0.197	4.1
Human serum 4	9.66	0.354	3.7	0.391	4.1
Human serum 5	18.1	0.576	3.2	0.607	3.4
PreciControl Varia 1	4.49	0.201	4.5	0.225	5.0
PreciControl Varia 2	12.5	0.360	2.9	0.529	4.2

Method comparison

a) A comparison of the Elecsys Folate III serum/plasma application, REF 08324174190 (**cobas e** 801 analyzer; y), with the Elecsys Folate III serum/plasma application, REF 07027290190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 142

Passing/Bablok[30]	Linear regression
y = 1.08x + 0.136	y = 1.07x + 0.161
τ = 0.942	r = 0.997

The sample concentrations were between 0.621 and 19.1 ng/mL (1.41 and 43.4 nmol/L).

b) A comparison of the Elecsys Folate III serum/plasma application, REF 08324174190 (**cobas e** 402 analyzer; y), with the Elecsys Folate III serum/plasma application, REF 08324174190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 143

Passing/Bablok[30]	Linear regression
y = 0.968x + 0.264	y = 0.961x + 0.322
τ = 0.913	r = 0.995

The sample concentrations were between 1.69 and 19.9 ng/mL (3.84 and 45.2 nmol/L).

Analytical specificity

The following cross-reactivities were found, tested with a folate concentration of approximately 4 ng/mL.

Cross-reactant	**Concentration tested ng/mL**	**Cross-reactivity %**
Amethopterin	750	0.6
Aminopterin	750	1.7
Folinic acid	750	0.5

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2. Folate RBC application

Test principle

Competition principle. Total duration of assay: 27 minutes.

Whole blood treated with anticoagulants (heparin or EDTA) is mixed with ascorbic acid solution and incubated for approximately 90 minutes at 20-25 °C. Lysis of the erythrocytes takes place, with liberation and stabilization of the intracellular folate. The resulting hemolysate sample is then used for subsequent measurement.

- 1st incubation: By incubating 15 µL of hemolysate sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as FOL 3.

PT1 Pretreatment reagent 1, 1 bottle, 7.3 mL:
Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT2 Pretreatment reagent 2, 1 bottle, 7.3 mL:
Sodium hydroxide 25 g/L.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Folate-binding protein~Ru(bpy)$_3^{2+}$, 1 bottle, 16.7 mL:
Ruthenium-labeled folate-binding protein 75 µg/L; human serum albumin (stabilizer); phosphate buffer 70 mmol/L, pH 5.5; preservative.

R2 Folate~biotin, 1 bottle, 13.9 mL:
Biotinylated folate 17 µg/L; human serum albumin (stabilizer); bis-tris propane buffer 100 mmol/L, pH 9.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

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

Response:

P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

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

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
Immediately call a POISON CENTER/ doctor.

P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[19,20]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The Elecsys Folate III kit can be used for both the folate serum/plasma application and the folate RBC application.

Both applications use the same reagents.

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Hemolysate prepared from whole blood treated with anticoagulants (Na-heparin or K_3-EDTA).

- *For the determination of folate in RBC*
  Determine hematocrit in whole blood samples and record the value.

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- *Preparation of the hemolysate sample*
  Mix 3.0 mL of Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %) and 100 µL of well-mixed whole blood, avoiding foam formation. Incubate with closed caps for 90 ± 15 minutes at 20-25 °C.

Stability:
If the hemolysate sample is prepared from fresh whole blood, it is possible to store the prepared hemolysate sample for 28 days at -20 °C (± 5 °C). Freeze only once. Analyze the sample promptly after thawing.

Whole blood storage prior to hemolysate preparation: 2 hours at 20-25 °C,[31] 24 hours at 2-8 °C, 28 days at -20 °C (± 5 °C; only EDTA blood). Freeze only once. If the whole blood sample was stored in one of these ways, the hemolysate sample must be used directly after preparation.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

If measurements cannot be carried out within 2 hours please store the hemolysate sample at -20 °C (± 5 °C).

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 08324247190, CalSet Folate, for 4 x 1.0 mL
- REF 05944317190, Folate RBC Hemolyzing Reagent kit for 4 x 200 mL, contains ascorbic acid
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

The well-mixed hemolysate sample is placed in the sample zone of the analyzer and recorded by entering the sample identification data. Complete determinations on the analyzer within 2 hours after finalizing the preparation of the hemolysate sample.

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This application has been standardized against the Elecsys Folate III assay (REF 04476433190)/RBC application.

The standardization of the folate RBC application includes the volume correction to account for the preparation of hemolysate sample (1:31 vol/vol).

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use commercially available whole blood control material.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

1. Whole blood folate (from hemolysate sample)

The standardization of the folate RBC application includes the volume correction to account for the preparation of hemolysate sample (1:31 vol/vol).

The analyzer automatically calculates the analyte concentration of each sample in nmol/L or ng/mL.

Conversion factors: nmol/L x 0.44 = ng/mL

ng/mL x 2.27 = nmol/L

2. RBC folate

To calculate the folate concentration in the erythrocyte fraction of the sample **(RBC folate)**, the predetermined sample specific hematocrit value must be taken into account using the following equation:

$$\text{RBC folate} = \frac{\text{analyzer result}}{\text{\% hematocrit}} \times 100$$

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 496 µmol/L or ≤ 29 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1000 IU/mL
IgG	≤ 1.6 g/dL
IgA	≤ 0.4 g/dL
IgM	≤ 1 g/dL

Criterion: For concentrations of 120-210 ng/mL the deviation is ≤ 21 ng/mL. For concentrations > 210 ng/mL the deviation is ≤ 10 %.

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Pharmaceutical substances
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drug was tested. No interference with the assay was found.

Special drug

Drug	Concentration tested U/mL
Erythropoietin	2000

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In rare cases, samples with low erythrocyte folate concentration, but high serum folate concentration can occur. In these cases, a correction of the folate concentration in erythrocytes by the serum folate concentration with the following equation is recommended*:

* expected values can be used as an indicator for high serum folate concentration

Corrected RBC folate concentration =

$$\text{RBC folate concentration} - \left(\text{serum folate concentration} \times \frac{100 - \%\ \text{hematocrit}}{\%\ \text{hematocrit}}\right)$$

Example

RBC folate concentration: 241 (ng/mL RBC);

serum folate concentration: 10.5 (ng/mL S);

hematocrit measured (%) = 45

Corrected RBC folate concentration =

$$241\ \text{ng/mL RBC} - \left(10.5\ \text{ng/mL S} \times \frac{100 - 45}{45}\right) = 228\ \text{ng/mL RBC}$$

Limits and ranges

Measuring range

120-620 ng/mL or 272-1407 nmol/L (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 120 ng/mL (< 272 nmol/L). Values above the measuring range are reported as > 620 ng/mL (> 1407 nmol/L). Values are not corrected for the sample hematocrit.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation:

Limit of Blank = 45 ng/mL (102 nmol/L)

Limit of Detection = 70 ng/mL (159 nmol/L)

Limit of Quantitation = 120 ng/mL (272 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 30 %.

It has been determined using low concentration folate samples.

Dilution

Hemolysate samples with folate concentrations above the measuring range can be diluted manually with Elecsys Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %). The recommended dilution is 1:2. The concentration of the diluted sample must be ≥ 265 ng/mL or ≥ 602 nmol/L. After manual dilution, multiply the results by the dilution factor 2.

Expected values

The values shown below were measured on samples from an apparently healthy population, using the Elecsys Folate III/RBC application. The values can be applied for the folate RBC application on all Elecsys and **cobas e** analyzers. The calculation is based on 290 sera (96 men, 194 women) from an European population. The age range was between 18 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal homocysteine values. The following values were obtained:

Whole blood folate (from hemolysate samples)					
	N	Median		2.5th-97.5th percentile	
		nmol/L	ng/mL	nmol/L	ng/mL
Europe	290	673	296	481-1212	212-534

The measured hematocrit value in this study showed a range from 37.1-46.1 %.

RBC folate (folate in erythrocyte fraction)					
	N	Median		2.5th-97.5th percentile	
		nmol/L	ng/mL	nmol/L	ng/mL
Europe	290	1657	730	1187-2854	523-1257

If pathologically low hematocrit values are considered for calculation of RBC folate in the erythrocyte fraction, elevated RBC folate concentrations may be observed. No medical conclusion should be based on the calculation considering hematocrit values in such cases. Instead, whole blood folate results (from hemolysate samples) and suitable expected values may be used.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents and hemolysate samples in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). Results are given as whole blood folate (from hemolysate sample). The following results were obtained:

cobas e 801 and **cobas e** 402 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean nmol/L	SD nmol/L	CV %	SD nmol/L	CV %
Hemolysate 1	345	13.0	3.8	14.0	4.1
Hemolysate 2	468	13.9	3.0	16.3	3.5
Hemolysate 3	572	15.2	2.7	18.8	3.3
Hemolysate 4	824	18.2	2.2	22.7	2.8
Hemolysate 5	1373	24.3	1.8	33.1	2.4

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cobas e 801 and **cobas e** 402 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Hemolysate 1	152	5.73	3.8	6.17	4.1
Hemolysate 2	206	6.14	3.0	7.17	3.5
Hemolysate 3	252	6.70	2.7	8.28	3.3
Hemolysate 4	363	8.01	2.2	10.0	2.8
Hemolysate 5	605	10.7	1.8	14.6	2.4

Method comparison

a) A comparison of the Elecsys Folate III RBC application, REF 08324174190 (**cobas e** 801 analyzer; y), with the Elecsys Folate III RBC application, REF 07027290190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 123

Passing/Bablok[30]	Linear regression
y = 1.04x - 12.3	y = 1.02x - 8.91
τ = 0.916	r = 0.992

The sample concentrations were between 132 and 618 ng/mL (300 and 1403 nmol/L).

b) A comparison of the Elecsys Folate III RBC application, REF 08324174190 (**cobas e** 402 analyzer; y), with the Elecsys Folate III RBC application, REF 08324174190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 142

Passing/Bablok[30]	Linear regression
y = 0.950x - 8.33	y = 0.947x - 8.34
τ = 0.923	r = 0.994

The sample concentrations were between 128 and 617 ng/mL (291 and 1401 nmol/L).

Analytical specificity

The following cross-reactivities were found, tested with a folate concentration of approximately 210 ng/mL.

Cross-reactant	**Concentration tested ng/mL**	**Cross-reactivity %**
Amethopterin	750	1.7
Aminopterin	750	2.0
Folinic acid	750	2.6

References

1 Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene 2014 Jan 1;533(1):11-20. doi: 10.1016/j.gene.2013.09.063.

2 Scaglione F, Panzavolta G. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica 2014 May;44(5):480-488. doi: 10.3109/00498254.2013.845705.

3 Reynolds EH. The neurology of folic acid deficiency. Handb Clin Neurol 2014;120:927-943. doi: 10.1016/B978-0-7020-4087-0.00061-9.

4 Bronsky J, Campoy C, Braegger C; ESPGHAN/ESPEN/ESPR/CSPEN working group on pediatric parenteral nutrition. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Vitamins. Clin Nutr 2018 Dec;37(6 Pt B):2366-2378. doi: 10.1016/j.clnu.2018.06.951.

5 Glader B. Anemias of inadequate production. In: Kliegman, Robert M.; Stanton, Bonita M.D.; Jenson, Hal B.; Behrman, Richard E, editors. Nelson textbook of pediatrics. 18th ed. Philadelphia: Saunders Elsevier; 2007. p. 2006-2018

6 WHO. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2012 (https://apps.who.int/iris/bitstream/handle/10665/75584/WHO_NMH_NHD_EPG_12.1_eng.pdf?sequence=1)

7 Snow CF. Laboratory Diagnosis of Vitamin B12 and Folate Deficiency: A Guide for the Primary Care Physician. Archives of Internal Medicine 1999;159(12):1289-1298.

8 Monsen AL, Refsum H, Markestad T, et al. Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19 years. Clin Chem 2003 Dec;49(12):2067-2075. doi: 10.1373/clinchem.2003.019869.

9 Kreusler P, Vogel M, Willenberg A, et al. Folate and Cobalamin Serum Levels in Healthy Children and Adolescents and Their Association with Age, Sex, BMI and Socioeconomic Status. Nutrients 2021 Feb 7;13(2):546. doi: 10.3390/nu13020546.

10 Soma-Pillay P, Nelson-Piercy C, Tolppanen H, et al. Physiological changes in pregnancy. Cardiovasc J Afr 2016 Mar-Apr;27(2):89-94. doi: 10.5830/CVJA-2016-021.

11 WHO antenatal care recommendations for a positive pregnancy experience. Nutritional interventions update: Multiple micronutrient supplements during pregnancy. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

12 Tuncalp Ö, Rogers LM, Lawrie TA, et al. WHO recommendations on antenatal nutrition: an update on multiple micronutrient supplements. BMJ Glob Health 2020 Jul;5(7):e003375. doi: 10.1136/bmjgh-2020-003375.

13 Yuan X, Han X, Zhou W, et al. Association of folate and vitamin B12 imbalance with adverse pregnancy outcomes among 11,549 pregnant women: An observational cohort study. Front Nutr 2022 Jul 25;9:947118. doi: 10.3389/fnut.2022.947118.

14 Milman N, Byg KE, Hvas AM, et al. Erythrocyte folate, plasma folate and plasma homocysteine during normal pregnancy and postpartum: a longitudinal study comprising 404 Danish women. Eur J Haematol 2006 Mar;76(3):200-5. doi: 10.1111/j.1600-0609.2005.00606.x.

15 Patti MA, Braun JM, Arbuckle TE, et al. Associations between folic acid supplement use and folate status biomarkers in the first and third trimesters of pregnancy in the Maternal-Infant Research on Environmental Chemicals (MIREC) Pregnancy Cohort Study. Am J Clin Nutr. 2022 Dec 19;116(6):1852-1863. doi: 10.1093/ajcn/nqac235.

16 Daly LE, Kirke PN, Molloy A, et al. Folate levels and neural tube defects. Implications for prevention. JAMA 1995 Dec 6;274(21):1698-1702. doi: 10.1001/jama.1995.03530210052030.

17 Chen MY, Rose CE, Qi YP, et al. Defining the plasma folate concentration associated with the red blood cell folate concentration threshold for optimal neural tube defects prevention: a population-based, randomized trial of folic acid supplementation. Am J Clin Nutr. 2019 May 1;109(5):1452-1461. doi: 10.1093/ajcn/nqz027.

18 Wick M, Pinggera W, Lehmann P. Clinical Aspects and Laboratory. Iron metabolism, Anemias. Springer Verlag, Wien, New York, 6th edition 2011:41-42.

19 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

20 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

21 Wu AHB. Tietz clinical guide to laboratory tests, 4th ed. St. Louis, Saunders/Elsevier 2006:608-609, 916-917.

22 Paricaud K, Moulis G, Combis MS, et al. Causes of protidemia above 100 g/L. Eur J Intern Med 2014;25:e123.

23 Filippatos TD, Liamis G, Christopoulou F, et al. Ten common pitfalls in the evaluation of patients with hyponatremia. Eur J Intern Med 2016;29:22-25.

24 Mailankody S, Landgren O. Monoclonal gammopathy of undetermined significance and Waldenström's macroglobulinemia. Best Pract Res Clin Haematol 2016;29:187-193.

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25 Morel P, Duhamel A, Gobbi P, et al. International prognostic scoring system for Waldenström macroglobulinemia. Blood 2009;113:4163-4170.

26 Rajkumar SV. Multiple Myeloma. Curr Probl Cancer 2009;33:7-64.

27 Gertz MA. Immunoglobulin light chain amyloidosis: 2016 update on diagnosis, prognosis, and treatment. Am J Hematol 2016;91:947-956.

28 Wu AHB. Tietz clinical guide to laboratory tests, 4th ed. St. Louis, Saunders/Elsevier 2006: 916-917, 925.

29 Pfeiffer CM, Johnson CL, Jain RB, et al. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988-2004. Am J Clin Nutr 2007;86:718-727.

30 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

31 Eijsden M, van der Wal MF, Hornstra G, et al. Can whole blood samples be stored over 24 hours without compromising stability of C-Reactive Protein, Retinol, Ferritin, Folic Acid and Fatty Acids in Epidemiology Research? Clin Chem 2005;51(1):230-232.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

Elecsys free PSA

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REF		Σ	SYSTEM
08828610190* 08828610214*	08828610500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
FPSA	10188

Please note

The Elecsys free PSA immunoassay should be used only with the Elecsys total PSA immunoassay to calculate the ratio (% fPSA) of free PSA (fPSA) to total PSA (tPSA). Use of another manufacturer's total PSA assay may result in an inappropriate population of patients selected for fPSA testing; and significantly different fPSA to tPSA ratios, cutoffs and prostate cancer probabilities than represented in the "Expected values" section of this insert. Ratios must be calculated using tPSA and fPSA results both obtained on either **cobas e** 402 or the **cobas e** 801 immunoassay analyzers.

The measured fPSA value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the fPSA assay method used. Free PSA values determined on patient samples by differing testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations.

Intended use

Immunoassay for the in vitro quantitative determination of free prostate-specific antigen in human serum and plasma.

This assay is indicated for measurement of fPSA in conjunction with the Elecsys total PSA assay to develop a ratio (% fPSA) of fPSA to tPSA. This ratio is useful when used in conjunction with the Elecsys total PSA test as an aid in distinguishing prostate cancer from benign prostatic conditions in men age 50 years or older who have a digital rectal examination (DRE) that is not suspicious for prostate cancer and an Elecsys total PSA value in the range 4 ng/mL to 10 ng/mL. Prostate biopsy is required for the diagnosis of prostate cancer.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Prostate-specific antigen (PSA) is a glycoprotein (molecular weight 30000-34000 daltons) having a close structural relationship to glandular kallikrein.

It has the function of a serine protease.[1]

The proteolytic activity of PSA in blood is inhibited by the irreversible formation of complexes with proteinase inhibitors such as alpha-1-antichymotrypsin (ACT) and alpha-2-macroglobulin.[2,3] In addition to being present in these complexes, PSA is also present in blood in the free form, but is proteolytically inactive.[3]

PSA tests lack sufficient sensitivity and specificity to be considered ideal or absolutely diagnostic for screening or early detection because PSA is not specific for prostate cancer.[4] PSA is organ specific, being produced primarily by prostatic secretory epithelium, but has long been known to be elevated in non-malignant conditions such as benign prostatic hyperplasia (BPH). A number of studies have found that the % free PSA was significantly lower in patients having prostate cancer than those with benign disease or normal controls.[5,6] The ratio fPSA/tPSA has subsequently been demonstrated to improve the sensitivity and specificity in patients with tPSA values in the "gray zone" of 4-10 ng/mL.[7,8]

An equimolar tPSA determination is the prerequisite for reliable ratios. In patients receiving therapy, particularly hormone withdrawal therapy, the fPSA/tPSA ratio cannot be utilized to differentiate prostate hyperplasia from cancer of the prostate. Combining tests from different manufacturers to determine tPSA and fPSA can produce erroneous values, since total PSA tests may be standardized by differing methods or detect free PSA to differing degrees.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 µL of sample, a biotinylated monoclonal PSA-specific antibody, and a monoclonal PSA-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as FPSA.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PSA-Ab~biotin, 1 bottle, 21.0 mL:
Biotinylated monoclonal anti-PSA antibodies (mouse) 2 mg/L; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 Anti-PSA-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 18.8 mL:
Monoclonal anti-PSA antibodies (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 100 mmol/L, pH 7.4; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

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P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95.

Stable for 8 hours at 20-25 °C, 5 days at 2-8 °C, 12 weeks at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 08851964190, free PSA CalSet, 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: The Elecsys free PSA assay has been standardized against the WHO Reference Standard 96/668 (100 % free PSA).

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or µg/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1500 IU/mL

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Criterion: For concentrations of 0.01-0.5 ng/mL the deviation is ± 0.06 ng/mL. For concentrations > 0.5 ng/mL the deviation is ± 10 %.

There is no high-dose hook effect at fPSA concentrations up to 15000 ng/mL.

Pharmaceutical substances
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special cancer drugs were tested. No interference with the assay was found.

Special cancer drugs

Drug	Concentration tested mg/L
Cyclophosphamide	1000
Cisplatin	225
5-Fluorouracil	500
Methotrexate	1000
Tamoxifen	50
Mitomycin	25
Carboplatin	1000
Etoposide	400
Flutamide	1000
Taxol	5.5
Doxorubicin	75

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.01-50 ng/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.01 ng/mL. Values above the measuring range are reported as > 50 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.01 ng/mL

Limit of Detection = 0.016 ng/mL

Limit of Quantitation = 0.018 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Not necessary due to the broad measuring range.

Expected values

A multicenter study was performed using samples from men (aged ≥ 50) referred to urologists for evaluation of prostate cancer (PCA). 1143 of the referred men had normal DRE that were not suspicious for prostate cancer (DRE normal cohort). Samples were evaluated using the Elecsys total PSA assay and Elecsys free PSA assay in parallel on the Elecsys 2010 immunoassay analyzer. A subset of these samples was evaluated on the MODULAR ANALYTICS E170 analyzer. No significant differences between the two platforms were observed.

All patients underwent a transrectal prostate biopsy. Of the 1143 men with normal DRE, 664 men had tPSA results between 4-10 ng/mL on the Elecsys 2010 analyzer (tPSA 4-10:DRE normal cohort). The ethnic composition of PSA 4-10:DRE normal cohort was 84.5 % Caucasian, 11.5 % Black non-Hispanic, 2.6 % Hispanic-Mexican, and 1.4 % other. The median age was 66 years. The distribution of fPSA, tPSA, and ratio fPSA/tPSA (% fPSA) values by biopsy result for this cohort is shown in table 1.

Table 1: PSA statistics by biopsy outcome (benign, malignant)

Elecsys 2010	Biopsy result	N	Mean ng/mL	Median ng/mL	Min. ng/mL	Max. ng/mL	Stand. error of mean
fPSA	Benign	463	1.19	1.11	0.26	4.14	0.02
	Malignant	201	1.00	0.92	0.34	2.39	0.03
	Total	664	1.13	1.06	0.26	4.14	0.02
tPSA	Benign	463	6.10	5.68	3.95	10.00	0.07
	Malignant	201	6.43	6.13	3.95	10.00	0.12
	Total	664	6.20	5.85	3.95	10.00	0.06
% fPSA	Benign	463	19.72	19.2	5.1	53.4	0.32
	Malignant	201	15.99	15.2	5.2	35.8	0.41
	Total	664	18.59	18.0	5.1	53.4	0.27

A comparison of the mean % fPSA for the benign and malignant biopsy groups indicated that the difference is significant.

The % fPSA result may be used in evaluating the need for prostate biopsy in one of two ways:

1. The relative risk of prostate cancer in individual men may be considered, or

2. Patients may be managed using a single cutoff.

1. Individual risk assessment

There is an increased probability of detecting PCA as the PSA level increases. Of interest is that in an urologically referred cohort there is a 12 % to 22 % risk of PCA in men whose tPSA is < 4.0 ng/mL. The tPSA range of 4-10 ng/mL has been described in references 6 and 7 as the diagnostic "gray zone". It is in this area that the % fPSA to tPSA ratio is of utility.

Table 2: Probability of detecting PCA on needle biopsy in urologically referred men with DRE results not suspicious for prostate cancer

tPSA ng/mL	Probability of PCA %	95 % confidence interval
< 4.0	17.1	12.5-21.6
4.0-10.0	30.3	26.8-33.8
> 10.0	49.1	42.5-55.7

The probability of finding PCA with tPSA in the gray zone (4-10 ng/mL) increases with increasing age and with decreasing fPSA/tPSA ratios - see table 3. The probabilities presented in table 3 were estimated from a loglinear model.

Table 3: Probability of finding PCA on needle biopsy by age in years and % fPSA on the Elecsys 2010 analyzer

Probability of finding PCA on needle biopsy by age in years (95 % confidence interval)			
% fPSA ratio	50-59	60-69	≥ 70
≤ 10	49.2 (12.4-86.9)	57.5 (17.9-89.3)	64.5 (30.4-88.3)
11-18	26.9 (5.7-68.9)	33.9 (8.6-73.7)	40.8 (15.8-71.7)
19-25	18.3 (3.5-57.9)	23.9 (5.4-63.4)	29.7 (10.1-61.1)
> 25	9.1 (3.1-23.7)	12.2 (4.7-28.1)	15.8 (9.0-26.1)

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2. Single cutoff

Alternatively, a single cutoff may be used for men in all age groups. Sensitivities (% of PCA detected) and specificities (% of biopsies avoided in men without PCA) for various % fPSA cutoffs are shown in table 4. A cutoff of 25 % results in the detection of 92.5 % of PCA and avoids unnecessary biopsy in 20.3 % of men without PCA. Virtually all (99 %) of PCA are detected with a cutoff of 30 %, but only 8.9 % of men without PCA are spared biopsy.

Table 4: Agreement with biopsy at various % fPSA cutoffs on the Elecsys 2010 analyzer

Benign biopsies			
free PSA %	Number of patients with negative biopsy identified at cutoff (total = 463)	Agreement at cutoff %	95 % confidence interval
23	141	30.5	26.3-34.9
25	94	20.3	16.7-24.3
27	65	14.0	11.0-17.5
30	41	8.9	6.4-11.8
53	1	0.2	0.0-1.2

Malignant biopsies			
free PSA %	Number of patients with positive biopsy identified at cutoff (total = 201)	Agreement at cutoff %	95 % confidence interval
23	173	86.1	80.5-90.5
25	186	92.5	88.0-95.8
27	192	95.5	91.7-97.9
30	199	99.0	96.5-99.9
53	201	100.0	98.2-100.0

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.0220	0.00141	6.4	0.00170	7.8
Human serum 2	0.150	0.00268	1.8	0.00338	2.3
Human serum 3	0.810	0.00986	1.2	0.0116	1.4
Human serum 4	2.12	0.0216	1.0	0.0307	1.4
Human serum 5	9.25	0.0731	0.8	0.115	1.2
Human serum 6	26.8	0.182	0.7	0.341	1.3
Human serum 7	45.6	0.418	0.9	0.569	1.2
Human serum 8	44.3	0.502	1.1	0.689	1.6
PC[b)] Tumor Marker1	0.951	0.0132	1.4	0.0152	1.6
cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
PC Tumor Marker2	9.66	0.132	1.4	0.166	1.7

b) PC = PreciControl

Method comparison

a) A comparison of the Elecsys free PSA assay, REF 08828610190 (**cobas e** 801 analyzer; y) with the Elecsys free PSA assay, REF 07027320190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 216

Passing/Bablok[9]

y = 0.995x + 0.005

τ = 0.985

The sample concentrations were between 0.010 and 49.4 ng/mL.

b) A comparison of the Elecsys free PSA assay, REF 08828610190 (**cobas e** 402 analyzer; y) with the Elecsys free PSA assay, REF 08828610190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured:189

Passing/Bablok[9]

y = 1.02x - 0.000

τ = 0.992

The sample concentrations were between 0.015 and 45.0 ng/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

Prostatic acid phosphatase (PAP) and ACT: none; PSA-ACT 0.7 %.

References

1 Henttu P, Vihko P. Prostate-specific Antigen and Human Glandular Kallikrein: Two Kallikreins of the Human Prostate. Ann Med 1994;26:157-164.

2 Tewari PC, Bluestein BI. Multiple forms of prostate specific antigen and the influences of immunoassay design on their measurement in patient serum. J Clin Ligand Assay, 18 1995;3:186-196.

3 Balk SP, Yoo-Joung K, Bubley GJ. Biology of Prostate-Specific Antigen. J Clin Oncol 2003;21(2):383-391.

4 Oesterling JE. Prostate-Specific Antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urology 1991(5);145:907-923.

5 Catalona WJ, Richie JP, Ahmann FR, et al. Comparison of digital rectal examination and serum prostate-specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. J Urology 1994;151(5):1283-1290.

6 Chen YT, Luderer AA, Thiel RP, et al. Using proportions of free to total prostate-specific antigen, age, and total prostate-specific antigen to predict the probability of prostate cancer. Urology 1996;47:518-524.

7 Thiel RP, Oesterling JE, Wojno KJ, et al. A multicenter comparison of the diagnostic performance of free prostate-specific antigen. Urology 1996;48(6A):45-50.

8 Luderer AA, Chen YT, Soriano TF, et al. Measurement of the proportion of free to total prostate-specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total prostate-specific antigen. Urology. 1995 Aug;46(2):187-94.

9 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

Elecsys free PSA

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
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Elecsys FSH

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REF		Σ	SYSTEM
07027346190	07027346500	300	**cobas e** 801

English

System information

Short name	ACN (application code number)
FSH	10114

Intended use

Immunoassay for the in vitro quantitative determination of follicle-stimulating hormone in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 801 immunoassay analyzer.

Summary

FSH (follicle stimulating hormone), together with LH (luteinizing hormone), belongs to the gonadotropin family. FSH and LH regulate and stimulate the growth and function of the gonads (ovaries and testes) synergistically.[1]

Like LH, TSH and hCG, FSH is a glycoprotein consisting of two subunits (α-and β-chains). Its molecular weight is approximately 32000 daltons.

In women FSH, in conjunction with LH, stimulates oestrogen secretion and ovulation.[2]

FSH and LH are released in pulses from the gonadotropic cells of the anterior pituitary. The levels of the circulating hormones are controlled by steroid hormones via negative feedback to the hypothalamus. In the ovaries FSH, together with LH, stimulates the growth and maturation of the follicle[2] and hence also the biosynthesis of estrogens in the follicles.

The FSH level shows a peak at mid-cycle, although this is less marked than with LH. Due to changes in ovarian function and reduced estrogen secretion, high FSH concentrations occur during menopause.[3]

In men, FSH serves to induce spermatogonium development.[2]

Determination of the FSH concentration is used in the elucidation of dysfunctions within the hypothalamus-pituitary-gonads system.

The determination of FSH in conjunction with LH is utilized for the following indications: congenital diseases with chromosome aberrations, polycystic ovaries (PCO), amenorrhea (causes), and menopausal syndrome. Depressed gonadotropin levels in men occur in azoospermia.[4]

The Elecsys FSH assay employs two different monoclonal antibodies specifically directed against human FSH. Cross-reactivity with LH, TSH, hCG, hGH, and hPL is negligible.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 24 µL of sample, a biotinylated monoclonal FSH-specific antibody, and a monoclonal FSH-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as FSH.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-FSH-Ab~biotin, 1 bottle, 21 mL:
Biotinylated monoclonal anti-FSH antibody (mouse) 0.5 mg/L, MES[b)] buffer 50 mmol/L, pH 6.0; preservative.

R2 Anti-FSH-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 13.9 mL:
Monoclonal anti-FSH antibody (mouse) labeled with ruthenium complex 0.8 mg/L, MES buffer 50 mmol/L, pH 6.0; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analyzer	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Elecsys FSH

cobas®

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 0.3 mIU/mL+ coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03032680122, FSH CalSet II, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** 801 analyzer

Additional materials for the **cobas e** 801 analyzer:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the Enzymun-Test FSH method. This in turn has been standardized against the 2nd IRP WHO reference standard 78/549.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in mIU/mL or in IU/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1900 mg/dL
Biotin	≤ 246 nmol/L or ≤ 60 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: For concentrations from 0.3-20 mIU/mL the deviation is ± 2.5 mIU/mL. For concentrations from 20-200 mIU/mL the deviation is ± 10 %.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at FSH concentrations up to 2000 mIU/mL.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.3-200 mIU/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.3 mIU/mL. Values above the measuring range are reported as > 200 mIU/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.1 mIU/mL

Limit of Detection = 0.3 mIU/mL

Limit of Quantitation = 1 mIU/mL

Elecsys FSH

cobas®

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Not necessary due to the broad measuring range.

Expected values

Studies with the Elecsys FSH assay have revealed the following FSH values:

Test subjects	N	FSH (mIU/mL)		
		Percentile		
		50th	5th	95th
Men	319	4.6	1.5	12.4
Women				
• Follicular phase	376	6.9	3.5	12.5
• Ovulation phase	56	12.3	4.7	21.5
• Luteal phase	349	3.6	1.7	7.7
• Postmenopause	181	67.0	25.8	134.8

LH/FSH quotient: Quotients have been calculated from the results obtained with the Elecsys LH assay and the Elecsys FSH assay in the samples of healthy women of child-bearing age. The following medians have been calculated:

Follicular phase: 0.82 (n = 315)

Luteal phase: 1.12 (n = 279)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 801 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	0.986	0.019	1.9	0.027	2.7
Human serum 2	9.91	0.159	1.6	0.324	3.3
Human serum 3	89.7	1.75	2.0	3.10	3.5
Human serum 4	131	3.61	2.8	4.09	3.1
Human serum 5	198	4.14	2.1	6.80	3.4
PC[c)] Universal 1	17.4	0.237	1.4	0.407	2.3
PC Universal 2	48.8	1.16	2.4	1.49	3.1

c) PC = PreciControl

Method comparison

A comparison of the Elecsys FSH assay, REF 07027346190 (**cobas e** 801 analyzer; y) with the Elecsys FSH assay, REF 11775863122 (**cobas e** 601 analyzer; x) gave the following correlations (mIU/mL):

Number of samples measured: 173

Passing/Bablok[5]	Linear regression
y = 0.997x - 0.050	y = 1.02x - 0.390
τ = 0.977	r = 1.00

The sample concentrations were between 0.957 and 186 mIU/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

Substance	Cross-reactivity %	Additive concentration mIU/mL
LH	0.022	5000
TSH	n.d.[d)]	5000
hCG	0.004	5000
hGH	n. d.	2000
hPL	n. d.	5000

d) n. d. = not detectable

References

1 Johnson MR, Carter G, Grint C, et al. Relationship between ovarian steroids, gonadotropin and relaxin during the menstrual cycle. Acta Endocrinol 1983;129/2:121-125.

2 Beastall GH, Ferguson KM, O'Reilly DSJ, et al. Assays for follicle stimulating hormone and luteinizing hormone: Guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem 1987;24:246-262.

3 Scott MG, Ladenson JH, Green ED, et al. Hormonal evaluation of female infertility and reproductive disorders. Clin Chem 1989;35:620-630.

4 Gudeloglu A, Parekattil SJ. Update in the evaluation of the azoospermic male. Clinics (Sao Paulo) 2013;68(Suppl 1):27-34.

5 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

Elecsys FSH

cobas®

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

Elecsys LH

cobas®

REF		Σ	SYSTEM
07027575190* 07027575214*	07027575500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
LH	10113

Intended use

Immunoassay for the in vitro quantitative determination of luteinizing hormone in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Luteinizing hormone (LH) measurements, performed with this assay, in human serum and plasma are used as an aid in diagnosis of the hypothalamic-pituitary-gonadal system, assessment of the primary cause of female and male infertility.

LH is a glycoprotein hormone with a heterodimeric structure, consisting of an α- and a β-subunit, where the α subunit is commonly shared with other hormones in the glycoprotein family. Hypothalamic gonadotropin-releasing hormone (GnRH) directs the pituitary to synthesize and secrete LH in a pulsatile pattern. LH together with follicle-stimulating hormone (FSH) control the functional activity of the gonads and synthesis of sex steroids.[1,2,3] Pituitary gonadotropin secretion is controlled by feedback from the gonadotropic hormones. In women, estrogen regulates LH secretion, and in men, testosterone regulates LH release.[1]

In women, LH acts together with FSH to regulate the menstrual cycle. The highest LH-concentrations occur during the mid-cycle peak to induce ovulation and to assist in the formation of corpus luteum promoting progesterone secretion.[1] In men, LH stimulates the development and functional activity of Leydig cells that produce testosterone.[1,4]

Determination of LH concentration is used in the elucidation of dysfunctions within the hypothalamus-pituitary-gonadal system. In women the determination of LH in conjunction with FSH is utilized for the indications such as congenital diseases with chromosome aberrations (e.g. Turner's syndrome) and infertility related conditions such as clarifying causes of amenorrhea, menopausal syndrome, polycystic ovary syndrome (PCOS). In men, measurement of LH is used for the assessment of male reproductive abnormalities leading to lowered levels of circulating testosterone (primary or secondary hypogonadism).[1,2,3,4]

The Elecsys LH assay employs two monoclonal antibodies specifically directed against human LH. The two specific antibodies used recognize particular conformations, with the biotinylated antibodies detecting an epitope constructed from both subunits whereas the antibody with the ruthenium complex[a)] label detects an epitope from the β-subunit. As a result, the Elecsys LH assay shows negligible cross-reactivity with FSH, TSH, hCG, hGH, and hPL.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 µL of sample, a biotinylated monoclonal LH-specific antibody, and a monoclonal LH-specific antibody labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

Reagents - working solutions

The **cobas e** pack is labeled as LH.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-LH-Ab~biotin, 1 bottle, 19.7 mL:
Biotinylated monoclonal anti-LH antibody (mouse) 2.0 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.

R2 Anti-LH-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 19.7 mL:
Monoclonal anti-LH antibody (mouse) labeled with ruthenium complex 0.3 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 0.3 mIU/mL+ coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03561097190, LH CalSet II, for 4 x 1.0 mL
- REF 09557423190, LH CalSet II, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the 2nd International Standard (NIBSC) 80/552.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 4 weeks when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

Use PreciControl Universal or other suitable controls for routine quality control procedures.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample either in mIU/mL or IU/L.

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1900 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: For concentrations from 0.3-20 mIU/mL the deviation is ± 2.5 mIU/mL. For concentrations from 20-200 mIU/mL the deviation is ± 10 %.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at LH concentrations up to 1150 mIU/mL.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

Elecsys LH

cobas®

0.3-200 mIU/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.3 mIU/mL. Values above the measuring range are reported as > 200 mIU/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.1 mIU/mL

Limit of Detection = 0.3 mIU/mL

Limit of Quantitation = 1 mIU/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Not necessary due to the broad measuring range.

Expected values

Studies with the Elecsys LH assay have revealed the following LH values:

Test subjects	N	LH mIU/mL		
		Percentile		
		50th	5th	95th
Men	322	4.0	1.7	8.6
Women				
• Follicular phase	316	5.9	2.4	12.6
• Ovulation phase	56	30.8	14.0	95.6
• Luteal phase	280	4.3	1.0	11.4
• Postmenopause	132	29.1	7.7	58.5

LH/FSH quotient: Quotients have been calculated from the results obtained with the Elecsys LH assay and the Elecsys FSH assay in the samples of healthy women of child-bearing age. The following medians have been calculated:

Follicular phase: 0.82 (n = 315)

Luteal phase: 1.12 (n = 279)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	0.992	0.021	2.2	0.023	2.3
Human serum 2	11.4	0.120	1.0	0.158	1.4
Human serum 3	63.4	0.631	1.0	0.707	1.1
Human serum 4	113	1.20	1.1	1.50	1.3
Human serum 5	194	1.80	0.9	2.30	1.2
PC[b)] Universal 1	10.7	0.120	1.1	0.177	1.6
PC Universal 2	51.4	0.655	1.3	1.08	2.1

b) PC = PreciControl

Method comparison

a) A comparison of the Elecsys LH assay, REF 07027575190 (**cobas e** 801 analyzer; y) with the Elecsys LH assay, REF 11732234122 (**cobas e** 601 analyzer; x) gave the following correlations (mIU/mL):

Number of samples measured: 146

Passing/Bablok[5]	Linear regression
y = 1.06x - 0.089	y = 1.04x + 0.228
τ = 0.992	r = 1.00

The sample concentrations were between 0.617 and 190 mIU/mL.

b) A comparison of the Elecsys LH assay, REF 07027575190 (**cobas e** 402 analyzer; y) with the Elecsys LH assay, REF 07027575190 (**cobas e** 801 analyzer; x) gave the following correlations (mIU/mL):

Number of serum samples measured:151

Passing/Bablok[5]	Linear regression
y = 0.958x + 0.045	y = 0.953x + 0.154
τ = 0.992	r = 1.00

The sample concentrations were between 0.448 and 194 mIU/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

Substance	**Additive concentration mIU/mL**	**Cross-reactivity %**
FSH	5000	0.005
TSH	5000	n. d.[c)]
hCG	5000	0.003
hGH	2000	n. d.
hPL	5000	n. d.

c) n. d. = not detectable

References

1 Holmes DT, Bertholf RL, Winter WE. Pituitary Function and Pathophysiology. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 55, p. 767-804.e10.

2 Nerenz RD, Boh B. Reproductive endocrinology and related disorders. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 58, p. 846-884.e11

3 Cole TJ. Hormones. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 38, p. 416-16.e14 .

4 Oduwole OO, Huhtaniemi IT, Misrahi M. The Roles of Luteinizing Hormone, Follicle-Stimulating Hormone and Testosterone in Spermatogenesis and Folliculogenesis Revisited. Int J Mol Sci. 2021;22(23):12735.

Elecsys LH

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5 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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www.roche.com
+800 5505 6606

Roche

Elecsys N-MID Osteocalcin

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REF		Σ	SYSTEM
			cobas e 411
12149133122	12149133500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 660
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 122

Intended use

Immunoassay for the in vitro quantitative determination of N-MID osteocalcin in human serum and plasma. The determination is used for the control of antiresorptives therapeutic efficiency, e.g. for patients with osteoporosis or hypercalcemia.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Osteocalcin, the most important non-collagen protein in bone matrix, is a bone-specific, calcium-binding protein which is dependent on vitamin K. It contains 49 amino acids and has a molecular weight of approximately 5800 Da. It contains up to three γ-carboxyglutamic acid residues (bone-GLA-protein, BGP). During bone synthesis osteocalcin is produced by the osteoblasts. Its production is dependent upon vitamin K (formation of γ-carboxyglutamic acid residues) and is stimulated by vitamin D3. After release from the osteoblasts, osteocalcin is not only assimilated into the bone matrix but also secreted into the blood stream. Accordingly, the serum (plasma) osteocalcin level is related to the rate of bone turnover and its measurement is useful in disorders of bone metabolism, e.g. osteoporosis in particular, but also in primary and secondary hyperparathyroidism.[1,2,3,4,5] Osteocalcin is therefore termed a bone turnover marker and is used for this purpose. By means of osteocalcin measurements it is possible to monitor therapy with antiresorptive agents (bisphosphonates or hormone replacement therapy, HRT) in, for example, patients with osteoporosis.[6,7,8] Both intact osteocalcin (amino acids 1-49) and the large N-MID fragment (amino acids 1-43) occur in blood. Intact osteocalcin is unstable due to protease cleavage between amino acids 43 and 44. The N-MID-fragment resulting from cleavage is considerably more stable.[4,9] The Elecsys N-MID Osteocalcin assay uses two monoclonal antibodies specifically directed against epitopes on the N-MID-fragment and the N-terminal-fragment. The assay hence detects the stable N-MID-fragment as well as the (still) intact osteocalcin. The test is non-dependent on the unstable C-terminal-fragment (amino acids 43-49) of the osteocalcin molecule and thus ensures constant measurement results under routine conditions in the laboratory.[10]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal N-MID osteocalcin-specific antibody, and a monoclonal N-MID osteocalcin-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as OSTEOC.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-N-MID Osteocalcin-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-N-MID Osteocalcin antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-N-MID Osteocalcin-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-N-MID Osteocalcin antibody (mouse) labeled with ruthenium complex 1.3 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Elecsys N-MID Osteocalcin

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Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Method comparison serum versus plasma, slope 0.9-1.10 + intercept within ≤ ± 1 ng/mL + coefficient of correlation ≥ 0.95.

Note: Avoid hemolysis! Erythrocytes contain proteases which degrade osteocalcin. It is recommended that blood be centrifuged immediately.

Stability of serum and heparinized plasma: 8 hours at 15-25 °C, 3 days at 2-8 °C, 3 months at -20 °C (± 5 °C). Freeze once only.

Stability of EDTA-plasma: 2 days at 15-25 °C, 3 days at 2-8 °C, 3 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 11972111122, N-MID Osteocalcin CalSet, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against in-house reference standards: osteocalcin in analyte-free human serum matrix.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Varia.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or µg/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1112 µmol/L or ≤ 65 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL

Elecsys N-MID Osteocalcin

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Compound	Concentration tested
Rheumatoid factors	≤ 2200 IU/mL

Criterion: Recovery within ± 2 ng/mL of initial value for samples ≤ 20 ng/mL and within ± 10 % of initial value for samples > 20 ng/mL.

Hemolysis interferes. Erythrocytes contain proteases which degrade osteocalcin.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at N-MID osteocalcin concentrations up to 4200 ng/mL.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.500-300 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 ng/mL. Values above the measuring range are reported as > 300 ng/mL (or up to 1500 ng/mL for 5-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 0.500 ng/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with N-MID osteocalcin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:5 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 50 ng/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The reference ranges are test-dependent. Completed studies (study protocol No. 9905) with the Elecsys N-MID Osteocalcin assay have revealed the following ranges in ng/mL:

	Number	N-MID osteocalcin	
		50th perc.	5-95th perc.
Healthy women			
• Premenopausal, > 20 yrs.	108	23	11-43
• Postmenopausal (no HRT)	102	27	15-46
Osteoporosis patients	120	27	13-48
Healthy men			
• 18- < 30 yrs.	183	40	24-70
• 30-50 yrs.	179	25	14-42
• > 50-70 yrs.	125	24	14-46

In patients with renal failure the osteocalcin values can be elevated, both directly, due to impaired clearance and indirectly, due to renal osteodystrophy.[11]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	6.01	0.085	1.4	0.186	3.1
Human serum 2	12.2	0.135	1.1	0.373	3.1
Human serum 3	35.6	0.601	1.7	1.06	3.0
Human serum 4	169	3.12	1.9	5.56	3.3
Human serum 5	8.11	0.091	1.1	0.159	2.0
PreciControl Varia 1	19.3	0.164	0.9	0.267	1.4
PreciControl Varia 2	93.2	1.01	1.1	1.65	1.8

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	6.11	0.056	0.9	0.120	2.0
Human serum 2	12.0	0.126	1.1	0.240	2.0
Human serum 3	34.5	0.361	1.1	0.677	2.0
Human serum 4	160	2.03	1.3	3.65	2.3
Human serum 5	7.49	0.066	0.9	0.107	1.4
PreciControl Varia 1	17.9	0.166	0.9	0.207	1.2
PreciControl Varia 2	85.9	0.755	0.9	1.12	1.3

Method comparison

A comparison of the Elecsys N-MID Osteocalcin assay (y) with a commercially available N-MID osteocalcin test (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 185

Passing/Bablok[12]	Linear regression
y = 1.29x - 2.79	y = 1.43x - 6.24
τ = 0.866	r = 0.987

The sample concentrations were between 10 and 210 ng/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

No cross-reactivity detectable for β-CrossLaps, parathyroid hormone, and bone-specific alkaline phosphatase.

References

1 Rosenquist C, Qvist P, Bjarnason N, et al. Measurement of a More Stable Region of Osteocalcin in Serum by ELISA with Two Monoclonal Antibodies. Clin Chem 1995;41(10):1439-1445.

2 Delmas PD, Wahner HW, Mann KG, et al. Assessment of bone turnover in postmenopausal osteoporosis by measurement of serum bone Gla-protein. J Lab Clin Med 1983;102(4):470-476.

Elecsys N-MID Osteocalcin

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3 Delmas PD, Wilson DM, Mann KG, et al. Effects of Renal Function on Plasma Levels of Bone Gla Protein. J Clin Endocrinol Metab 1983;57(5):1028-1030.

4 Garnero P, Grimaux M, Seguin P, et al. Characterisation of Immunoreactive Forms of Human Osteocalcin Generated In Vivo and In Vitro. J Bone Miner Res 1994;9(2):255-264.

5 Epstein S. Bone-Derived Proteins. Trends Endocrinol Metab 1989;1:9-14.

6 Chen J-T, Hosoda K, Hasumi K, et al. Serum N-Terminal Osteocalcin is a Good Indicator for Estimating Responders to Hormone Replacement Therapy in Postmenopausal Women. J Bone Miner Res 1996;11(11):1784-1792.

7 Ravn P, Christensen JO, Baumann M, et al. Changes in Biochemical Markers and Bone Mass After Withdrawl of Ibandronate Treatment: Prediction of Bone Mass Changes During Treatment. Bone 1998;22(5):559-564.

8 Brown JP, Malaval L, Chapuy MC, et al. Serum bone GLA protein: A specific marker for bone formation in postmenopausal osteoporosis. Lancet 1984:1091-1093.

9 Gundberg CM. Biology, Physiology, and Clinical Chemistry of Osteocalcin. J Clin Ligand Assay 1998;21(2):128-138.

10 Garnero P, Delmas PD. New Developments in Biological Markers for Osteoporosis. Calcif Tissue Int 1996;59(1):2-9.

11 Masters PW, Jones RG, Purves DA, et al. Commercial assays for serum osteocalcin give clinically discordant results. Clin Chem 1994;40(3):358-363.

12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

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CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

Elecsys Progesterone III

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REF		Σ	SYSTEM
07027699190* 07027699214*	07027699500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System Information

Short name	ACN (application code number)
PROG 3	10045

Intended use

Immunoassay for the in vitro quantitative determination of progesterone in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Progesterone measurements, performed with this device, in human serum and plasma are used as an aid in diagnosis of female fertility.

The gestagen progesterone is a steroid hormone which is mainly formed in the cells of the corpus luteum in the ovaries and during pregnancy in the placenta. Minor sources of progesterone are the adrenal cortex in both sexes and the testes in men.

The progesterone concentration correlates with the development and regression of the corpus luteum. Whereas progesterone is barely detectable in the follicular phase of the female cycle, a rise in the progesterone level is observed one day prior to ovulation. Increased progesterone synthesis occurs during the luteal phase. In the second half of the cycle pregnanediol is excreted in urine as the main degradation product of progesterone.[1]

Progesterone brings about the conversion of the uterine mucosa into a tissue rich in glands (luteal phase), in order to prepare for the intrauterine implantation of the fertilized ovum.[1] During pregnancy, progesterone maternal serum concentrations increase, inhibiting the contraction of the myometrium and maintaining pregnancy. In the mammary gland, progesterone (together with estrogens) promotes the proliferation, secretion and disposition of the alveoli. [1,2,3,4]

Progesterone determination is used in fertility diagnosis to detect ovulation and assess the luteal phase.[5]

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (12 µL) with a progesterone-specific biotinylated antibody, immunocomplexes are formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and an progesterone derivative labeled with a ruthenium complex[a)], the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as PROG 3.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-progesterone-Ab~biotin, 1 bottle, 21.0 mL:
Biotinylated monoclonal anti-progesterone antibody (recombinant, sheep) 30 ng/mL, phosphate buffer 25 mmol/L, pH 7.0; preservative.

R2 Progesterone-peptide~$Ru(bpy)_3^{2+}$, 1 bottle, 18.8 mL:
Progesterone (of vegetable origin) coupled to a synthetic peptide labeled with ruthenium complex, 2 ng/mL; phosphate buffer 25 mmol/L, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Elecsys Progesterone III

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Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 0.1 ng/mL + coefficient of correlation ≥ 0.95.

Stable for 1 day at 20-25 °C, 5 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 07092547190, Progesterone III CalSet, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- REF 03028542122, Diluent Estradiol/Progesterone, 2 x 22 mL sample diluent
- REF 09762582190, Elecsys Progesterone Diluent, 2 x 22 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: The Elecsys Progesterone III assay is traceable via ID-GC/MS (isotope dilution gas chromatography/mass spectrometry) to highly purified progesterone by weight analogously to BCR-348R and ERM-DA347.[6]

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 8 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, ng/mL or in µg/L).

Conversion factors: nmol/L x 0.314 = ng/mL (µg/L)

ng/mL x 3.18 = nmol/L

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 923 µmol/L or ≤ 54 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 200 mg/dL
Biotin	≤ 123 nmol/L or ≤ 30 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7 g/dL
IgA	≤ 0.4 g/dL
IgM	≤ 1 g/dL

Criterion: Recovery within ± 10 % of initial value for samples > 2 ng/mL, ± 15 % for samples > 0.5 to 2 ng/mL and ± 0.2 ng/mL for samples ≤ 0.5 ng/mL.

Visibly turbid samples give a false low result.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. Of these, only phenylbutazone at therapeutic dosage levels showed interference with the assay (progesterone values depressed).

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In addition, the following special drug was tested. No interference with the assay was found.

Special drug

Drug	Concentration tested mg/L
Clomiphene citrate	100

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.159-191 nmol/L or 0.05-60 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.159 nmol/L or < 0.05 ng/mL. Values above the measuring range are reported as > 191 nmol/L or > 60 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.080 nmol/L (0.025 ng/mL)

Limit of Detection = 0.159 nmol/L (0.05 ng/mL)

Limit of Quantitation = 0.636 nmol/L (0.2 ng/mL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

Dilution

Samples with progesterone concentrations above the measuring range can be diluted with Elecsys Progesterone Diluent or a suitable human serum with a low analyte concentration. The recommended dilution is 1:10. The concentration of the diluted sample must be ≥ 3.18 nmol/L (≥ 1 ng/mL).

After manual dilution, multiply the result by the dilution factor.

Depending on the biological variance of the diluted patient sample and the human serum matrix used for production of Elecsys Progesterone Diluent, lower recovery of diluted samples may be observed.

Expected values

The expected ranges were determined by testing specimens drawn from 147 apparently healthy males, 142 apparently healthy, post-menopausal women over the age of 50, and from 416 apparently healthy pregnant women between the ages of 18 and 50 (137 in the first trimester, 140 in the second trimester, and 139 in the third trimester). The expected range for healthy women was determined by collecting blood at multiple time points of one menstrual cycle from 85 apparently healthy subjects with a natural menstrual cycle that were not taking any hormonal contraceptives. A menstrual cycle was defined as the phase between two subsequent menstrual bleedings. Cycle length (29 days) and day of ovulation (day 15) were standardized to account for variation in cycle length within the study population and to enable determination of expected values for further sub-phases. Only ovulatory menstrual cycles were used for value analysis. Based on a central 90 % interval, the following ranges were obtained:

Test subjects	N	5th percentile nmol/L (90 % CI*)	Median nmol/L (90 % CI)	95th percentile nmol/L (90 % CI)
Healthy men	147	< 0.159 (< 0.159-< 0.159)	< 0.159 (< 0.159-< 0.159)	0.474 (0.442-0.614)
Healthy postmenopausal women				
• Postmenopause	142	< 0.159 (< 0.159-< 0.159)	< 0.159 (< 0.159-< 0.159)	0.401 (0.343-0.480)
Healthy pregnant women				
• 1st trimester	137	35.0 (24.8-40.4)	76.3 (73.1-82.3)	141 (126-156)
• 2nd trimester	140	80.8 (71.3-86.2)	151 (144-159)	265 (251-315)
• 3rd trimester	139	187 (167-218)	342 (328-372)	679 (607-826)

* CI = confidence interval

Healthy women Cycle Phase	N **	5th percentile nmol/L (90 % CI)	Median nmol/L (90 % CI)	95th percentile nmol/L (90 % CI)
Follicular	85	< 0.159 (< 0.159-< 0.159)	0. 212 (0.186-0.244)	0.616 (0.584-0.897)
Ovulation	81	0.175 (< 0.159- 0.301)	1.81 (1.57-2.26)	13.2 (6.19-19.4)
Luteal	85	13.1 (8.34-15.6)	28.8 (26.4-31.4)	46.3 (43.2-64.8)

** N = number of patients contributing to the data in this menstrual cycle phase (not number of samples); differences in N per phase are due to cycle standardization procedure

Healthy women Cycle Sub-Phase	N	5th percentile nmol/L (90 % CI)	Median nmol/L (90 % CI)	95th percentile nmol/L (90 % CI)
Early follicular	78	< 0.159 (< 0.159-< 0.159)	0.38 (0.32-0.47)	1.03 (0.802-2.58)
Intermediate follicular	83	< 0.159 (< 0.159-< 0.159)	0.21 (0.168-0.252)	0.7 (0.619-3.44)
Late follicular	84	< 0.159 (< 0.159-< 0.159)	0.188 (< 0.159-0.234)	0.688 (0.579-12.3)
Ovulation	79	< 0.159 (< 0.159-0.171)	1.59 (1.09-1.85)	7.49 (5.91-19.4)
Early luteal	85	7.53 (4.66-9.53)	22.6 (20.3-24.9)	48 (39.9-54.1)
Intermediate luteal	81	15.2 (5.39-22.9)	39.2 (36.4-44.4)	66.5 (63.4-78.5)
Late luteal	84	1.71 (< 0.159-3.46)	18.2 (16.6-20.5)	43.1 (38.5-72.3)

Test subjects	N	5th percentile ng/mL (90 % CI)	Median ng/mL (90 % CI)	95th percentile ng/mL (90 % CI)
Healthy men	147	< 0.050 (< 0.050-< 0.050)	< 0.050 (< 0.050-< 0.050)	0.149 (0.139-0.193)

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Test subjects	N	5th percentile ng/mL (90 % CI)	Median ng/mL (90 % CI)	95th percentile ng/mL (90 % CI)
Healthy postmenopausal women				
• Postmenopause	142	< 0.050 (< 0.050-< 0.050)	< 0.050 (< 0.050-< 0.050)	0.126 (0.108-0.151)
Healthy pregnant women				
• 1st trimester	137	11.0 (7.81-12.7)	24.0 (23.0-25.9)	44.3 (39.6-48.9)
• 2nd trimester	140	25.4 (22.4-27.1)	47.5 (45.2-50.0)	83.4 (78.9-99.1)
• 3rd trimester	139	58.7 (52.7-68.5)	107 (103-117)	214 (191-260)

Healthy women Cycle Phase	N	5th percentile ng/mL (90 % CI)	Median ng/mL (90 % CI)	95th percentile ng/mL (90 % CI)
Follicular	85	< 0.050 (< 0.050-< 0.050)	0.067 (0.058-0.077)	0.193 (0.183-0.282)
Ovulation	81	0.055 (< 0.050-0.095)	0.568 (0.493-0.709)	4.14 (1.94-6.09)
Luteal	85	4.11 (2.62-4.9)	9.04 (8.29-9.84)	14.5 (13.5-20.3)

Healthy women Cycle Sub Phase	N	5th percentile ng/mL (90 % CI)	Median ng/mL (90 % CI)	95th percentile ng/mL (90 % CI)
Early follicular	78	< 0.050 (< 0.050-< 0.050)	0.119 (0.1-0.147)	0.323 (0.252-0.809)
Intermediate follicular	83	< 0.050 (< 0.050-< 0.050)	0.066 (0.053-0.079)	0.22 (0.194-1.08)
Late follicular	84	< 0.050 (< 0.050-< 0.050)	0.059 (< 0.050-0.074)	0.216 (0.182-3.87)
Ovulation	79	< 0.050 (< 0.050-0.0547)	0.499 (0.342-0.581)	2.35 (1.86-6.09)
Early luteal	85	2.36 (1.46-2.99)	7.11 (6.37-7.8)	15.1 (12.5-17)
Intermediate luteal	81	4.76 (1.69-7.19)	12.3 (11.4-13.9)	20.9 (19.9-24.6)
Late luteal	84	0.537 (< 0.050-1.09)	5.72 (5.2-6.43)	13.5 (12.1-22.7)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
			Repeatability		
Sample	Mean		SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%
Human serum 1	0.172	0.054	0.035	0.011	20.7
Human serum 2	2.10	0.659	0.089	0.028	4.2
Human serum 3	9.64	3.03	0.264	0.083	2.7
Human serum 4	70.0	22.0	0.789	0.248	1.1
Human serum 5	170	53.5	1.84	0.579	1.1
PreciControl U[b)]1	23.9	7.52	0.480	0.151	2.0
PreciControl U2	49.6	15.6	0.712	0.224	1.4

b) U = Universal

cobas e 402 and **cobas e** 801 analyzers					
			Intermediate precision		
Sample	Mean		SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%
Human serum 1	0.172	0.054	0.076	0.024	43.9
Human serum 2	2.10	0.659	0.130	0.041	6.2
Human serum 3	9.64	3.03	0.321	0.101	3.3
Human serum 4	70.0	22.0	1.18	0.372	1.7
Human serum 5	170	53.5	2.86	0.898	1.7
PreciControl U1	23.9	7.52	0.677	0.213	2.8
PreciControl U2	49.6	15.6	0.989	0.311	2.0

Method comparison

a) A comparison of the Elecsys Progesterone III assay, REF 07027699190 (**cobas e** 801 analyzer; y) with the Elecsys Progesterone III assay, REF 07092539190 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 153

Passing/Bablok[7]	Linear regression
y = 0.984x + 0.001	y = 0.981x + 0.086
τ = 0.985	r = 0.999

The sample concentrations were between 0.050 and 59.0 ng/mL.

b) A comparison of the Elecsys Progesterone III assay, REF 07027699190 (**cobas e** 402 analyzer; y) with the Elecsys Progesterone III assay, REF 07027699190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 167

Passing/Bablok[7]	Linear regression
y = 1.04x + 0.117	y = 1.05x - 0.040
τ = 0.982	r = 0.999

The sample concentrations were between 0.066 and 54.3 ng/mL.

Analytical specificity

For the Elecsys Progesterone III assay, the following cross-reactivities were found at the respective additive concentration, tested with progesterone concentrations of approximately 0.3 ng/mL and 5 ng/mL:

Substance	**Additive concentration ng/mL**	**Cross-reactivity %**
Androstenediol	4000	0.001
Androstenedione	80	0.107
Aldosterone	1000	0.003

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Substance	Additive concentration ng/mL	Cross-reactivity %
Allopregnanolone	2000	0.347
Corticosterone	200	0.921
Cortisol	20000	0.006
Danazol	100000	0.001
DHEA-S	16000	n. d.[c)]
Norgestrel	1000	0.011
Estradiol	400	n. d.
Ethisterone	1000	0.001
Ethynodiol diacetate	1000	n. d.
Medroxyprogesterone	5000	0.004
Norethindrone	1000	0.004
Norethindrone acetate	1000	0.008
Testosterone	2000	0.069
21-Deoxycortisol	2000	0.067
11-Deoxycorticosterone	600	3.92
11-Deoxycortisol	6000	0.015
5-α-Dihydrotestosterone	20	n. d.
5-β-Dihydroprogesterone	240	0.366
Pregnenolone	16000	0.410
Pregnanolone	2000	0.145
Medroxyprogesterone acetate	1000	0.003
6α-Methylprednisolone	1000	0.003
17α-Hydroxypregnenolone	2000	0.009
17α-Hydroxyprogesterone	2000	0.066
20α-Hydroxy-4-pregnen-3-one	250	0.86

c) n. d. = not detectable

References

1 Nerenz RD, Boh B. Reproductive endocrinology and related disorders. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 58, p.846-884.e11.

2 Ku CW, Allen JC Jr, Lek SM, et al. Serum progesterone distribution in normal pregnancies compared to pregnancies complicated by threatened miscarriage from 5 to 13 weeks gestation: a prospective cohort study. BMC Pregnancy Childbirth. 2018 Sep 5;18(1):360. doi: 10.1186/s12884-018-2002-z.

3 Macias H, Hinck L. Mammary gland development. Wiley Interdiscip Rev Dev Biol. 2012;Jul-Aug;1(4):533-57. doi: 10.1002/wdev.35.

4 Schock H, Zeleniuch-Jacquotte A, Lundin E, et al. Hormone concentrations throughout uncomplicated pregnancies: a longitudinal study. BMC Pregnancy Childbirth. 2016 Jul 4;16(1):146. doi: 10.1186/s12884-016-0937-5.

5 Fertility problems: assessment and treatment. London: National Institute for Health and Care Excellence (NICE); 2017 Sep.

6 Thienpont L, Siekmann L, Lawson A, et al. Development, Validation and Certification by Isotope Dilution Gas Chromatography-Mass Spectrometry of Lyophilized Human Serum Reference Materials for Cortisol (CRM 192 and 193) and Progesterone (CRM 347 and 348). Clin Chem 1991;37(4):540-546.

7 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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