HSV Type 1 recombinant gG1

IgG – ELISA

Enzyme immunoassay for the qualitative determination of IgG-class antibodies against HSV Type 1
in human serum
Only for in-vitro diagnostic use

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Product Number: HSV1G0500 (96 determinations)

CONTENTS

1. INTRODUCTION

Herpes simplex is an enveloped DNA virus (150-200 nm in diameter) belonging to the alpha-herpesviridae. Based on antigenic, biochemical and biologically differences it can be divided into two serotypes, HSV-1 and HSV-2. Man is the only known natural host and source of the virus. HSV Type 1 typically causes oral herpes, while HSV-type 2 typically affects the genital area. Most of the time, HSV-1 and HSV-2 are inactive, or "silent", and cause no symptoms, but some infected people have "outbreaks" of blisters and ulcers. Once infected with HSV, people remain infected for life. Herpes simplex viruses are amongst the most common infectious agents of man, and either HSV type appears to be capable of infecting similar body sites. A high percentage of the adult population is seropositive (appr. 90% HSV-1, in dependence on the socio-economic status 10-30% HSV-2). Primary HSV-1 infection usually occurs in early childhood (6 to 18 months of age). HSV-2 usually produces mild symptoms, and most people have no recognized symptoms. Persons at risk are children with inherited T-cell deficiencies and patients who are immunosup-pressed because of infection (e.g. HIV), transplantation, or cancer therapy.

Species	Disease	Symptoms	Mechanism of Infection
HSV-1 (Herpes labialis)	Oral herpes Primary: Herpetic gingivostomatitis complications are e.g. herpetic keratitis and encephalitis Recurrent form: herpes labialis	Multiple vesicles in the oral mucous membranes and fever blisters on the mouth or face	Transmission by droplet infection

The presence of virus resp. infection may be identified by

- Microscopy: CPE, IF
- PCR
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The NovaTec HSV 1 IgG recombinant gG1 ELISA allows the detection of HSV 1 infection in presence of antibodies to HSV 2.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgG-class antibodies against HSV Type 1 is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with HSV Type 1 recombinant antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured HSV Type 1-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of HSV Type 1-specific IgG antibodies in the specimen. Sulfuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **HSV Type 1 Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with HSV Type 1 recombinant antigen gG1; vacuum sealed, in resealable aluminium foil.
- **IgG Sample Diluent** ***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.
- **Stop Solution**: 1 bottle containing 15 ml sulfuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **HSV Type 1 anti-IgG Conjugate**:** 1 bottle containing 20 ml of peroxidase labelled rabbit antibody to human IgG; colored red, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **HSV Type 1 IgG Positive Control***:** 1 bottle containing 2 ml; colored yellow; ready to use; red cap.
- **HSV Type 1 IgG Negative Control***:** 1 bottle containing 2 ml; colored yellow; ready to use; blue cap.

* contains 0.01 % Thimerosal after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (20…25°C) before starting the test run!

6.1. Coated snap-off Strips

The ready to use breakapart snap-off strips are coated with recombinant gG1 antigen. Recombinant gG1 protein of HSV-1 show one band in SDS electrophoresis corresponding to gG1 which is found at approximately 43 kD. Store at 2...8°C. The strips are vacuum sealed. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the dessiccant supplied and stored at 2...8 °C; stability until expiry date.*

6.2. HSV Type 1 anti-IgG Conjugate

The bottle contains 20ml of a solution with anti-human-IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert red dye. The solution is ready to use. *Store at 2...8°C. After first opening stability until expiry date when stored at 2…8°C.*

6.3. Controls

The bottles labelled with Positve and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2...8°C. *Store at 2...8°C. After first opening stability until expiry date when stored at 2…8°C.*

6.4. IgG Sample Diluent

The bottle contains 100ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8°C. *Store at 2...8°C. After first opening stability until expiry date when stored at 2…8°C.*

6.5. Washing Solution (20xconc.)

The bottle contains 50ml of a concentrated buffer, detergents, stabilizers and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for at least four weeks if stored at 2...8°C. *Crystals in the solution disappear by warming up to 37 °C in a water bath.*

6.6. TMB Substrate Solution

The bottle contains 15ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. *The solution should be colourless or have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be discharged. Store at 2...8°C. After first opening stability until expiry date when stored at 2…8°C.*

6.7. Stop Solution

The bottle contains 15ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.
Store at 2...8°C. After first opening stability until expiry date when stored at 2…8°C.

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum samples with this assay. If the assay is performed within 24 hours after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex. *Positive and negative controls are ready to use and must not be diluted.*

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
2 wells	(e.g. B1+C1)	for the negative control and
1 well	(e.g. D1)	for the positive control.

It is recommended to determine controls and patient samples in duplicate, if necessary.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl HSV Type 1 anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.** *Do not expose to direct sunlight.*
7. Repeat step 4.

8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue color developed during the incubation turns into yellow.*

    Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2..
11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop solution.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance value **lower than 0.100.**
- **Negative control** in B1 and C1: Absorbance value **lower than 0.300.**
- **Positive control** in D1: Absorbance value equal to or greater than the cut-off value.

9.2. Calculation of Results

The cut-off is calculated by addition of 0.20 absorbance units to the measured absorption of the mean value of the two negative control determinations.

Example: 0.12 OD neg. control + 0.14 OD neg. control = 0.26 ÷ 2 = 0.13

Cut-off = absorbance mean value of the negative control + 0.20

Cut-off = 0.13 + 0.20 = 0.33

9.3. Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative
→ **grey zone**
It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

9.3.1. Results in NovaTec Units

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec-Units = NTU]}$$

Example: $\frac{1.786 \times 10}{0.33} = 54$ *NTU (NovaTec Units)*

Cut-off :	10	NTU
Grey zone:	9-11	NTU
Negative:	<9	NTU
Positive:	>11	NTU

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

Interassay	n	Mean	Cv (%)
Pos. Serum	17	1.26	8.8

Intraassay	n	Mean	Cv (%)
Pos. Serum	7	1.28	7.4

10.3. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 98 %.

10.4. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 98 %.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.
In immunocompromised patients and newborns serological data only have restricted value.

12. PRECAUTIONS AND WARNINGS

- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING:	Thimerosal is toxic! Do not swallow. Avoid contact with skin and mucous membranes!
WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulfuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13. LITERATURE

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14. ORDERING INFORMATION

Prod. No.:	HSV1G0500	HSV Type 1 IgG-recombinant gG1 ELISA (96 Determinations)

SCHEME OF THE ASSAY

HSV Type 1 recombinant gG1 IgG-ELISA

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Sample (diluted 1+100)
Negative control	-	100µl	-	-
Positive control	-	-	100µl	-
Sample (diluted 1+100)	-	-	-	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well three times with 300µl of washing solution				
Conjugate	-	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution				
TMB Substrate	100µl	100µl	100µl	100µl
Incubate for exactly 15 min at room temperature in the dark				
Stop Solution	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)				

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