

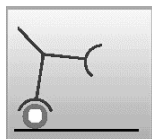
* In case of early herpes infection (4-6 days of disease) the result can be negative. In case of clinical presentations a performance of repeated testing in 10-14 days is recommended.

LIMITATION OF THE PROCEDURE

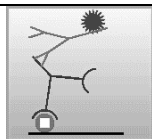
During diagnostics results should be interpreted in conjunction with the patient's clinical history, symptomatology, the results of others diagnostics procedures as well.

OVERVIEW OF PROCEDURE

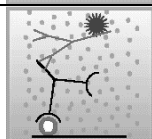
- Wash once with washing solution
- Dispense 100 µl of diluted investigated specimens and controls in wells
- Incubate for 60 min at 37°C
- Wash 4 times with washing solution



- Dispense 100 µl of conjugate solution in wells
- Incubate for 30 min at 37°C
- Wash 6 times with washing solution



- Dispense 100 µl TMB substrate in wells
- Incubate for 30 min at room temperature (colouring)
- Stop the reaction by adding 100 µl stop-reagent
- Read the optical density at 450/620 nm



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Diaproph Med
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DIA-HSV 2 -IgG

Enzyme immunoassay for the qualitative and semiquantitative detection of IgG class antibodies against Herpes Simplex Virus 2 type

96 tests

Product code: T-17G07C

EXAMPLE FORM

INTENDED USE

Enzyme immunoassay kit is intended for the qualitative and semiquantitative detection of IgG antibodies to Herpes Simplex Virus 2 type in human serum or plasma.

INTRODUCTION

Herpes Simplex Virus (HSV) belongs to the alphaherpesviridae and is an envelope DNA virus 150-200 nm in diameter. It is divided into two serotypes HSV-1 and HSV-2, which have common a group specific nucleocapsid antigen and different type specific antigens. HSV-1 mainly affects face skin and oral mucous membrane, HSV-2 typically causes genital herpes.

According to WHO data about 20% of adult population is infected by HSV-2. This type of virus in 80 % cases is the cause of genital and neonatal herpes. The genital infection caused by HSV-2, in comparison with HSV-1, more often recurs and is taking its serious pathology. HSV-2 can cause an oncogenic cell transformation, miscarriage (pregnant women) and congenital malformation in development of fetus. All those are reasons of unfavourable prognosis of neonatal herpes infection of newborns. In most cases neonatal herpes infection is taking its atypical form and in 20% cases – asymptotically that causes to rapid spreading of virus. Taking it into consideration during diagnostics of HSV infection the confirmation of HSV-2 presence in organism is required in a laboratory diagnostics using serological technique for investigation.

Specific IgG antibodies appear at 10-14 days after infection and remain for life, so serological techniques allow to reveal both active viral and latent infection.

PRINCIPLE OF PROCEDURE

Main components of the kit are an microelisa strips (immunosorbent) and conjugate. Microelisa strips are coated with gG2 recombinant antigen – an analogue of type specific antigen of HSV-2. The conjugate is presented by monoclonal antibodies to human IgG conjugated with the horseradish peroxidase.

When investigated specimens of human plasma or serum are placed into wells IgG antibodies to HSV-2 are bound to the antigen on the solid phase. Specific IgG to HSV-2 are detected with the conjugate - monoclonal antibodies-HRP.

After washing non-bound components the developer solution (substrate buffer and chromogen TMB) is added into each well.

The peroxidase reaction is stopped by adding the stop-reagent then the absorbance in wells is measured at 450/620 nm, the optical density of mixture is proportional to the concentration of IgG antibodies in serum or plasma specimens.

For evaluation of antibody titre changing in dynamics, the result of test can be expressed in DIA Units (DU).

STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8°C. The kit must not be frozen. Shelf life of the kit is 12 months.

KIT REAGENTS

For *in vitro* diagnostic use.

Each kit contains:

№	Reagents	Presentation
1	Stock washing solution (№1) Phosphate buffer, containing 2.2% Triton X100.	2 bottles 2 × 25 ml
2	Microelisa strips 12 strips per plate each with 8 wells coated with recombinant HSV2 gG2 antigen	1 plate
3	Solution for sera dilution (№3) Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.05% sodium azide, 0.01% benzoic acid.	2 bottle 2 × 30 ml
4	Solution for conjugate dilution (№4) Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01 sodium azide, 0.02% thimerosal, 0.01% benzoic acid.	1 bottle 1 × 15 ml
5	Substrate buffer Citrate-phosphate buffer, containing 0.016% hydrogen peroxide.	1 bottle 1 × 8 ml
6	Chromogen TMB Solution containing 0.03% 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 8 ml

- Add to each well 100 µl of the **stop-reagent** (maintain the same pipetting sequence and rate used for TMB substrate addition).
 - Read the absorbance at 450/620 nm using a dual wavelength microplate reader within 5 minutes after stopping the reaction.
- An absorbance may be measured at 450 nm (single wavelength) against a blank well; in that case include an empty well in the run.*

Interpretation of results

PC – absorbance of the positive control

NC – mean absorbance of the negative control

- Calculate the mean absorbance for negative controls.

Test run is valid if \overline{NC} is not higher than 0.2 optical units (OU).

Exclude any NC, which is higher than 0.2 OU or if it is more than twice exceed the \overline{NC} , and consider the remaining control (NC).

Test run is valid if PC is not lower than 0.6 OU.

- Calculate **Cut-off** value.

$$\text{Cut-off} = \overline{NC} + 0.20$$

- Determine the grey zone:

Grey zone is the zone with sample absorbance within the following ranges

$$\text{Cut-off} - 10\% \leq OD \leq \text{Cut-off}$$

- The sample is considered **negative** if the specimen absorbance is below the grey zone.
- The sample is considered **positive** if the specimen absorbance is greater than the cut-off.
- Specimens with absorbance values lying within the grey zone are considered **indeterminate** and in this case they should be retested in duplicate. If the repeated results are equivocal, another specimen should be collected in 10-14 days for retesting with the original sample. If result is laying in the same ratio values, this sample is considered negative.

Evaluation of results in DIA Units

- DIA Units serves for evaluation of antibody titre changing in dynamics:

$$DU = \frac{OD_{\text{serum}} \times 10}{\text{cut-off}}$$

Results are interpreted as follows:

> 10 DU	positive
9 – 10 DU	indetermined
< 9 DU	negative*

- TMB substrate (solution for chromogen preparation + chromogen TMB) is to be colourless. Appearance of colouring after dilution is evidence of unavailability for using and solution is to be replaced. The solution is to be prepared in clean plastic ware or clean glassware. The reagent is to be kept in dark.
- Prevent the direct light to fall on the working surface during ELISA procedure.
- Use a new tip for brining specimens in wells.
- Never use the same trough for distribution conjugate and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing must be performed strictly according to the instructions, as insufficient plate washing leads to incorrect results.

Use automatic washer*, as recommended; in case of its absence or faulty work – use multi-channel pipette for washing.

Follow this procedure in each washing:

- aspirate the wells contents completely into a waste flask;
- then fill the wells completely with washing solution (not less than 350 µl per well) avoiding overflow of buffer from one well to another;
- aspirate completely.

Make sure that no fluid remains on the top and the bottom of the strips and strip holder after the last aspiration (e. g. by blotting with absorbent tissue).

* Contact our company for further information on the different types of washers validated by our technical services.

Test procedure

- Fit the strip holder with required number of **strips**.
- Wash once all wells with the **washing solution** (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Add 100 µl of preliminary diluted specimens (1:50) and controls (1:50) to wells as follows:
 - well A1: 100 µl of the **positive control**.
 - well B1, C1: 100 µl of the **negative control**.
 - well D1, E1, etc.: 100 µl of the **specimens**.
- Cover the plate with adhesive film and incubate at 37°C for 60 minutes.
- Aspirate the contents of all wells and wash the plate with the **washing solution** 4 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Add 100 µl of the **conjugate solution** to each well.
- Cover the plate with adhesive film and incubate at 37°C for 30 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Add to each well 100 µl of the **TMB substrate**.
- Cover the plate with adhesive film and incubate at 18-25°C for 30 minutes in the dark.

№	Reagents	Presentation
7	Conjugate (concentrate 11x) anti-human IgG monoclonal antibodies conjugated with a horseradish peroxidase. Preservatives: 0.01% thimerosal.	1 vial 1 × 1.5 ml
8	Negative control Inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to HSV-2, HIV and HCV. Preservatives: 0.1% sodium azide.	1 vial 1 × 0.3 ml
9	Positive control Human serum reactive for antibodies to HSV-2. Inactivated by heating. Preservatives: 0.01 % MIT, 0.02% BND.	1 vial 1 × 0.2 ml
13	Stop-reagent 0.5 M sulphuric acid solution.	1 bottle 1 × 15 ml
14	Adhesive film	3 items

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- vial for reagents preparation (glass or plastic);
- graduated cylinder (1000 ml);
- absorbent paper;
- sodium hypochlorite solution or other accepted disinfectant;
- sodium bicarbonate;
- ethanol, 70°;
- automatic single-channel pipettes (e.g. 5-40, 20-200, 200-1000 µl) with disposable tips;
- automatic multi-channel pipettes (50-300 µl) with disposable tips;
- incubator, 37±1°C;
- microwell wash system*;
- microwell reader* (with dual wavelength 450/620);
- biohazard waste containers for potentially contaminated materials.

* Contact our company for further information on the equipment validated by our technical services.

SAFETY PRECAUTIONS AND WARNINGS

- Use a new tip for pipetting specimens in wells.
- All reagents included in the kit are intended for "in vitro" diagnostic use.
- Wear disposable gloves when handling reagents and samples and thoroughly wash hands after handling them.
- Do not pipette by mouth.
- Human origin material used in the preparation of the negative and positive controls. The positive control has been inactivated by heating. The positive and negative

controls were tested and found nonreactive for hepatitis B surface antigen (HBsAg), antibodies HCV and HIV (HIV-1, HIV-2).

- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.
- Any equipment directly in contact with specimens and reagents as well as the washing solution be considered as contaminated products and treated as such.
- Avoid spilling samples or solution containing samples.
- Spills must be treated with ethyl alcohol 70°. If the contaminating fluid is an acid, spill must be neutralized with sodium bicarbonate and dried with absorbent paper. The materials used for cleaning must be discarded in a contaminated residue container.
- Samples and reagents of human origin, as well as, contaminated material and products must be discarded after decontamination:
 - Either by immersion solid wastes in sodium hypochlorite at a final concentration of 5%, liquid wastes in sodium hypochlorite at a final concentration of 1% during 30 min.
 - Or by autoclaving at 121°C during 2 hours. The best method of inactivating of HIV, HBV, and HCV is an autoclaving.
 - DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE.
- Do not forget neutralize acid solutions before autoclave.
- Avoid any contacts substrate solution, chromogen and stop-reagent with skin and mucous covers.
- The negative and positive controls contain sodium azide as a preservative. Sodium azide may react with laboratory plumbing forming copper or lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a huge quantity of water if solutions containing azide are disposed of the sink after inactivation.

SPECIMEN PREPARATION

Serum or plasma specimens are to be stored at 2-8°C during 72 hours. If necessary these specimens may be frozen (not more than two freezing-thawing procedures are allowed) at temperature below –20°C.

All specimens containing aggregates and visible suspended particles are to be clarified by centrifugation.

Specimens with sodium azide, hemolysis, hyperlipidemiae or bacterial contamination may not be used in the ELISA procedure.

ASSAY PROCEDURE

Reagents and specimens should be at room temperature (18-25°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use.

Reagents preparation (for 8 wells)

Microelisa strips

Open the bag and remove the strip plate. Return unused strips in the pack. Reseal the pack and store at 2-8°C.

The strips are stable for 4 weeks at 2-8°C after opening the pack.

Washing solution

Check **Stock washing solution (№1)** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37°C.

Dilute the 4 ml of the **stock washing solution (№1)** with 180 ml of the distilled or de-ionised water, shake intensively to dissolve any crystals that may be present.

Washing solution is stable for 10 days at 2-8°C.

Serum dilution (specimens and controls)

Investigated serum samples, positive and negative controls are preliminarily diluted (1:50) with the **solution for sera dilution**. For example, 10 µl of serum is added to 500 µl of the **solution for sera dilution**. Dilution of specimens should be performed before use. *Carefully repipette the mixture.*

Note: Dilution of serum and controls is not to be straight performed in wells of the strip plate. It can be run in separate vessels.

Conjugate solution

Dilute 100 µl of the (11x) **conjugate** with 1 ml of the **solution for conjugate dilution (№4)** (1:10) in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

Conjugate solution is stable for 6 hours at 2-8°C.

TMB substrate

In order to prepare **TMB substrate**, dilute 0.5 ml of the **chromogen TMB** with 0.5 ml of the **solution for chromogen preparation (№5T)** in a clean vial (1:1). Mix well. TMB substrate must be colourless before use.

The TMB substrate must be kept away from light and no solutions contact with metals or metal ions is allowed.

The solution has to be prepared before use.

TMB substrate is stable for 2 weeks at room temperature (18-25°C) if kept in the dark.

Procedural notes

Authenticity of results depends on correct execution following instructions:

- Reagents should not be used beyond the expiry date shown on the package label.
- Reagents should not be mixed from different lots during performing test.
- Reagents and samples should be at room temperature (18-25°C) before testing begins. Return the reagents to 2-8°C after use.
- The temperature in room where performing analysis should be in the range 18-25°C.
- It should accurately dissolve reagents avoiding its contamination.
- Do not perform the test in the presence of reactivity vapours (for example, from sodium hypochlorite, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
- Use glass vessels thoroughly washed and rinsed with deionized water or use disposable ones.
- Do not allow drying contents of wells on all stages of procedure.
- Enzyme reaction is sensitive to metal ions, so avoid contacting with metal elements.