

- Positive samples in one or two wells are to be considered positive with DIA-HIV-Ag/Ab;
 - Negative samples in two wells are to be considered negative with DIA-HIV-Ag/Ab.
- All specimens, which are positive during retesting, should be performed in confirmatory test kits. Due to the high sensitivity of DIA-HIV-Ag/Ab at early seroconversion stage it is recommended to include a sensitive HIV antigen assay in confirmatory testing, for example DIA-HIV-p24.

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Diaproph Med
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DIA-HIV-Ag/Ab

Fourth generation ELISA test kit for the detection specific antibodies to HIV 1/2 and HIV antigen (p24)

96 tests

Product code: T-1507

INTENDED USE

Enzyme immunoassay kit is intended for the detection of HIV-1 antigen and specific antibodies (IgG, IgM, IgA) to human immunodeficiency virus (HIV ½) in human serum and plasma.

EXAMPLE FORM

INTRODUCTION

Infectious disease caused by Human Immunodeficiency Virus (HIV) spreads in a short space of time all over the world. The terminal stage of HIV-infection was assumed the name the acquired immunodeficiency syndrome (AIDS) characterized by strongly depressed immunity. At the present moment it has been isolated two types of virus – HIV1 and HIV-2. Today there are not preventives for HIV-infection and existent methods of antiviral therapy do not guarantee complete recovery from AIDS. At the early stage of HIV-seroconversion antibodies to main HIV p24 protein can be detected with ELISA on average at 3-6 weeks after infection (75 % infectious persons). The more immunogenic glycoproteins are gp160, gp120 and gp41, antibodies usually appear late to them. First it is usually appeared antibodies IgM to core proteins that is presents in serum during circulation of viral particulates in human blood. The main way of HIV transmission is sexual contacts, contamination of blood and certain blood product, transmission virus from mother to her foetus or child. The main role on the path to limitation its disease belong to actions that direct at identifying potentially infectious units of donated blood or plasma. The more popular practice of serological investigation is the detection of antibodies to HIV with ELISA. Today considerable improvements of the ELISA sensitivity were achieved and a time between infection with HIV and a time when antibodies can be detected (“seroconversion window”) was reduced. Further steps in direction of reducing such “window” are possible in case of incorporation of HIV p24 antigen detecting parallel with HIV antibodies that allows revealing of infected people as soon as possible.

PRINCIPLE OF PROCEDURE

Main components of the test kit are microplate and immunoenzyme conjugates. The microplate is polystyrene plate coated with monoclonal antibodies to HIV antigen (p24) and the mixture of recombinant proteins env-1 and env-2, which are analogues of HIV-1 and HIV-2 antigens, accordingly. The immunoenzyme conjugates are presented as the following: polyclonal antibodies to HIV p24 antigen labelled with biotin; recombinant antigens env-1 and env-2 conjugated with a horseradish peroxidase and streptavidin peroxidase conjugate.

When investigated infected specimen of human plasma or serum is placed into wells of the immunoplate, p24 antigen is bound to both specific antibodies on the solid phase and polyclonal antibodies to p24 labelled with biotin composing the conjugate No1; and specific antibodies to HIV are bound to both recombinant antigens env-1 and env-2 on the solid phase and antigens composing the conjugate No 1 forming the complexes of antigen-antibody. Immune complexes with anti-p24 specific antibodies and p24 antigen are detected by the streptavidin peroxidase conjugate No2. The developer substrate solution (the peroxidase substrate - hydrogen peroxide and TMB chromogen) is added into wells after washing of unbound components. The reaction is stopped by adding the 0.5 M sulphuric acid, then the optical density of the mixture in wells is measured at 450/620 nm. The colour intensity in wells depends on the presence of anti-HIV specific antibodies or p24 antigen.

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:

No.	Reagents	Presentation
1	Washing solution concentrate Phosphate buffered solution, Tween-20	2 bottles 2 × 25 ml
2	Microplate Microplate is coated with anti- p24 monoclonal antibodies and recombinant antigens env-1, env-2	1 plate (96 tests)
3	Conjugate concentrate No1 Mixture of rabbit affinity purified polyclonal antibodies to p24 labelled with biotin and purified recombinant antigens env-1 and env-2 conjugated with horseradish peroxidase in the stability solution (blue coloured) Preservatives: 0.04 % 2-methyl-4-isothiazolin-3-one	1 vial 1 × 0.8 ml
4	Conjugate concentrate No2 Streptavidin conjugated with horseradish peroxidase in the stability solution (red coloured) Preservatives: 0.04 % 2-methyl-4-isothiazolin-3-one	1 vial 1 × 1.5 ml
5	Conjugate diluent No1 PBS, skimmed milk, 0.5% Triton X-100 (blue coloured) Preservatives: 0.01 % thimerosal, 0.01% benzoic acid	1 bottle 1 × 8 ml

- Dispense 100 µl of **conjugate solution No2** per well, cover the plate with adhesive film and incubated at 18-25 °C for 15 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). After finishing washing procedure dry the plate by slight tapping upside-down on the absorbent paper.
- Prepare **TMB substrate** according to 1.4.
- Dispense 100 µl **TMB substrate** into all wells.
- Cover the plate with adhesive film and incubate at 18-25 °C for 30 minutes in the darkness.
- Add into each well 100 µl of **stop-reagent** to stop colour reaction (maintain the same pipetting sequence and rate used for TMB substrate dispensing).
- Read the absorbance at 450/620 nm using using a dual wavelength microplate reader within 5 minutes after stopping reaction.
Absorbance may be measured at 450 nm (single wavelength) against a blank well; for that purpose include an empty well in the run.

Results

Calculation of the results

NC – absorbance of the negative control

PC_{Ab} – absorbance of the positive control Ab

PC_{Ag} – absorbance of the positive control Ag

\overline{NC} – mean absorbance of the negative control

- Calculate the mean absorbance (\overline{NC}) for wells with negative control.
Test performance is considered correct if the optical densities for positive controls PC_{Ab} and PC_{Ag} are not less than 0.6 OU; and \overline{NC} is not more than 0.15 OU. If one out of three values of the negative control is more than 0.15 OU or differs from \overline{NC} more than twice (in other words not comply following requirements $0.5 \times \overline{NC} \leq NC \leq \overline{NC} \times 2$), those value is not taking into consideration. In this case \overline{NC} is calculated with two NC.
- **Cut-off** is calculated: Cut-off = $\overline{NC} + 0.2$.
- **“Grey zone”** is the zone with sample absorbance within the range
Cut-off – 10 % ≤ OD ≤ Cut-off.

Interpretation of the results

- Results are considered **negative** with the DIA-HIV-Ag/Ab if the optical density of investigated samples is below the “Grey zone”.
- Results are considered **positive** by the DIA-HIV-Ag/Ab if the optical density of investigated samples is greater than Cut-off .
- Samples, those values of the optical density are in “Grey zone”, are considered **indeterminate** and should be retested with caution in duplicate.
- Positive samples revealed during test performance should be retested not less than in two wells of the test kit:

- 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.
- TMB substrate (substrate buffer + TMB solution) 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 solution and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing is to 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;
- allow to soak during 40-60 seconds;
- aspirate completely.

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

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

Test procedure

- Open the seal pack and remove the plate. Fit the stripholder with required number of **strips**. Return unused strips in the pack. Reseal the pack and return to 2-8 °C. The strips are stable for 4 weeks at 2-8 °C after opening the seal pack.
- Prepare the **washing solution** according to item 1.1.
- Prepare the **conjugate solution No1** (blue coloured) according to item 1.2.
- Distribute in the wells as follows:
 - well A1: 100 µl of **positive control Ab**.
 - well B1: 100 µl of **positive control Ag**.
 - wells C1, D1, E1: 100 µl of **negative control**.
 - the rest wells: 100 µl of investigated samples.
- Distribute 30 µl of **conjugate solution No1** in all the wells. If it is possible homogenise the mixture by careful shaking the microplate for 15-20 seconds.
- Cover the strips with adhesive film and incubate at 40-42 °C for 1 hour.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). After finishing washing procedure dry the plate by slight tapping upside-down on the absorbent paper.
- Prepare the **conjugate solution No2** (red coloured) according to item 1.3.

No.	Reagents	Presentation
6	Conjugate diluent No2 PBS, 0.05% Tween-20 (red coloured) Preservatives: 0.02% 2-methyl-4-izotiazoline-3-one, 0,01% benzoic acid.	1 bottle 1 × 15 ml
7	Substrate buffer 0,01% benzoic acid, phosphate-citric buffer, 0.016 % hydrogen peroxide	1 bottle 1 × 7 ml
8	TMB solution 0.03 % 3,3',5,5'-tetramethylbenzidine	1 bottle 1 × 7 ml
9	Positive control Ab Human serum reactive for antibodies to HIV. Inactivated by heating and β-propiolacton. Preservatives: 0.04% 2-methyl-4-izotiazoline-3-one	1 vial 1 × 1.4 ml
10	Positive control Ag Heating inactivated human serum containing purified HIV antigen (p24) and nonreactive with HBsAg, antibodies to HIV, hepatitis C and Treponema pallidum Preservatives: 0.04% 2-methyl-4-izotiazoline-3-one	1 vial 1 × 1.4 ml
11	Negative control Heating inactivated human serum nonreactive with HIV antigen (p24), HBsAg, antibodies to HIV, hepatitis C and Treponema pallidum Preservatives: 0.1% sodium azide.	2 vial 2 × 2.0 ml
12	Stop-reagent	1 bottle 1 × 15 ml
13	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, 40-42°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 Ab-positive control has been inactivated by heating and β -propiolacton. The negative control has been tested and found nonreactive for hepatitis B surface antigen (HBsAg), antibodies to HCV and antibodies to HIV (HIV-1, HIV-2), however for the purpose of additional protection treated with heating.
- 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 buffer, TMB solution and stop-reagent with skin and mucous covers.
- The negative control contains 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 endured at room temperature (18-25°C) at least 30 minutes before beginning the assay performance and can remain at room temperature during testing. After use they are to be returned in temperature regime at 2-8°C.

Reagents preparation (for 8 wells)

1.1 Washing solution

Check **Washing solution concentrate** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37 °C. The content of the one bottle is intensively shaken. 2 ml of the concentrate is taken from the bottle and dissolved in 60 ml of the distilled or deionised water.

Washing solution is stable for 5 days at 2-8 °C.

1.2 Conjugate solution No 1

Dilute 50 μ l **Conjugate concentrate No1** (11x) (blue colour) with 0.5 ml **Conjugate diluent No1** (blue colour), this procedure is to be performed in a clean vial. The content of the vial is thoroughly mixed avoiding foaming.

Conjugate solution has to be prepared before use.

1.3 Conjugate solution No 2

Dilute 100 μ l **Conjugate concentrate No2** (11x) (red colour) with 1.0 ml **Conjugate diluent No2** (red colour), this procedure is to be performed in a clean vial. The content of the vial is thoroughly mixed avoiding foaming.

Conjugate solution has to be prepared before use.

1.4 TMB substrate

To prepare **TMB substrate**, mix 0.5 ml of **TMB solution** and 0.5 ml of **Substrate buffer**, this procedure is performed in a clean vial. The content of the vial is thoroughly mixed. TMB substrate has to be prepared before use and is to be colourless before use.

The TMB substrate is to be kept away from light and not to be contacted with any metals or metal ions.

The substrate solution is to be prepared before use.

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

Procedure 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.