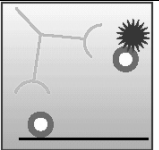
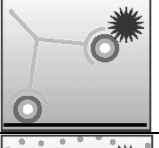
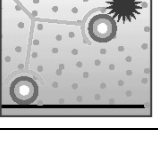


In all investigated concentrations of above mentioned factors in samples, the sensitivity of DIA-HIV 1/2 did not become deteriorate. However, during the specificity studies in the case of negative serum samples containing of bilirubin in the concentration of 75-110 µM/L, the tendency to increasing of OD/CO value (up to 0.4-0.8) was observed. Therefore, a potential false positive results must be taken into account when testing samples with concentration of bilirubin exceeding above mentioned. In the case of obtaining of repeated positive results in DIA-HIV 1/2 for samples with heightened bilirubin level, confirmatory methods must be used (Western Blot, NAT).

Other factors in investigated concentrations (triglycerides, total proteins, hemoglobin, E.coli antibodies and also bilirubin in the concentration lower than 75 µM/L) do not influence on OD/CO ratio and the assay specificity.

It should be considered the specificity of DIA-HIV 1/2 for serum samples from pregnant women is 0.26 % lower than for blood donors (99.3 % and 99.56 % correspondingly).

## OVERVIEW OF PROCEDURE

<ul style="list-style-type: none"> <li>• Dispense 60 µl of conjugate solution and 30 µl both control specimens and investigated sera into wells</li> </ul>	
<ul style="list-style-type: none"> <li>• Incubate for 90 min at 37 °C (forming complex antigen-antibody with conjugate)</li> <li>• Wash 8 times with washing solution</li> </ul>	
<ul style="list-style-type: none"> <li>• Dispense 100 µl TMB substrate into wells</li> <li>• Incubate for 30 min at room temperature (colouring)</li> <li>• Stop the reaction by adding 100 µl stop-reagent</li> <li>• Read the optical density at 450/620 nm</li> </ul>	

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**Diaproph Med**  
Diagnostics Prophylaxis Medicine

# DIA-HIV 1/2

**Enzyme immunoassay for the detection  
of antibodies to Human Immunodeficiency  
Virus types 1 and 2 (HIV-1 and HIV-2)**

96 test

Product code: T-0107C

## INTENDED USE

Enzyme immunoassay kit is intended for the detection of antibodies to human immunodeficiency virus (HIV) types 1 and 2 in human serum and plasma.

## INTRODUCTION

Infectious disease caused by Human Immunodeficiency Virus (HIV) has been spreading all over the world and the number of people living with HIV has been growing every year. HIV is transmissible infection that can be mainly acquired through sexual contact between persons infected with HIV, blood products contaminated with HIV, improper disinfected medical instruments and transmission from mother to foetus / child. The terminal stage of HIV-infection is an acquired immunodeficiency syndrome (AIDS) characterized by a strongly depressed immunity. Today, two types of virus – HIV1 and HIV-2 have been isolated.

At the early stage of HIV-seroconversion, antibodies to main core HIV protein (p24) can be detected with third generation ELISA on average at 3-6 weeks after infection. More immunologically relevant glycoproteins are gp160, gp120 and gp41, antibodies to them usually appear late. First, IgM antibodies to core protein usually appears, which are present in serum during circulation of viral particles in human blood.

The main available tool heading toward a spreading limitation of HIV-infection belongs to actions directed to identification of HIV antibodies in donated human serum or plasma. The most popular practice and necessary to guarantee the safety of the blood supply is screening methods basing on enzyme immunoassay (ELISA).

## PRINCIPLE OF THE ASSAY

DIA-HIV 1/2 enzyme-linked immunosorbent assay is based on one-step "sandwich" principle. There is used a solid phase (microplate strips) coated with a mixture of recombinant HIV-specific proteins (HIV-1 – env-1 [gp120, gp41], gag-1 [p24, p17] and HIV-2 – env-2 [gp 36]) and conjugate (a mixture of recombinant proteins HIV-1 [env-1 gag-1] and HIV-2 [env-2] bound to a horseradish peroxidase).

The principle of procedure represents a one-step procedure with the simultaneous incubation of investigated sample and conjugate. When investigated human plasma or serum specimens and conjugate are placed into wells, HIV-specific antibodies presented in specimen are bound to both recombinant antigens on a solid phase and conjugate antigens forming antigen-

antibodies complexes. Unspecifically bound components are removed by washing steps. The substrate buffer (hydrogen peroxide) and TMB solution are added to wells after washing unbound components.

The solution is coloured in case of a presence of peroxidase conjugate in complexes. The enzyme reaction is stopped by adding of the stop-reagent (sulphuric acid), then the optical density (OD) is determined in a spectral photometer at 450/620 nm.

### STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8 °C. Freezing of the kit is not allowed.

The shelf life of the kit is 15 months.

### KIT REAGENTS

For *in vitro* diagnostic use.

Each kit contains:

No	Reagents	Presentation
1	<b>Microplate strips</b> 12 strips per plate each with 8 breakable wells coated with recombinant HIV-1 and HIV-2 antigens.	1 plate
2	<b>Washing solution concentrate</b> Phosphate buffer, containing 2.2 % Triton X100	2 bottles 2 × 25 ml
3	<b>Positive control</b> Immunoglobulins from human serum reactive for antibodies to HIV-1, HIV-2. Inactivated by heating. Preservatives: 0.04 % 5-bromo-5-nitro-1, 3-dioxan, 0.1 % 2-methyl-4-isothiazolin-3-one.	1 vial 1 × 1.0 ml
4	<b>Negative control</b> Heating inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to HIV and hepatitis C virus (HCV). Preservatives: 0.1 % sodium azide.	1 vial 1 × 1.8 ml
5	<b>Conjugate concentrate (11x)</b> Recombinant HIV-1 and HIV-2 antigens bound to a horseradish peroxidase (HRP). Preservatives: 0.1 % 2-methyl-4-isothiazolin-3-one.	1 vial 1 × 1.5 ml
6	<b>Conjugate diluent</b> Phosphate buffer, containing powdered milk. Preservatives: 0.01 % thimerosal.	1 bottle 1 × 15 ml
7	<b>TMB solution</b> Solution containing 0.03 % 3,3',5,5'-tetramethylbenzidine	1 bottle 1 × 8 ml
8	<b>Substrate buffer</b> Citrate-phosphate buffer, containing 0.016 % hydrogen peroxide	1 bottle 1 × 8 ml
9	<b>Stop-reagent</b> 0.5M sulphuric acid solution	1 bottle 1 × 15 ml
10	<b>Adhesive film</b>	3 items

### ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- vial for reagents preparation (glass or plastic);

HIV-HCV co-infection, 54 serum samples from patients with different stages of HIV-infection / AIDS. All above mentioned samples were detected in DIA-HIV ½ as positive.

- Reactivity of DIA-HIV ½ to the detection of antibodies against different HIV subtypes was confirmed by using the following serum samples: subtype O (5), A (5), G (4), C (6), D (5), E (4), F (1). The detection of antibodies to HIV O group is assured with cross-reactivity env-1 M subtype. All serum samples in DIA-HIV ½ were positive.

The sensitivity of the assay was 100 %. Study tasting shown DIA-HIV ½ met the current state-of-the art level according to the Common Technical Specifications for the Directive 98/79/EC.

### Specificity and cross-reactivity

Diagnostic specificity of DIA-HIV ½ made 99.56 % during investigations of 2891094 non-selected blood donors. For purposes of evaluation of analytical specificity, 23432 potential cross-reactive and interfering samples were tested. Mentioned samples were obtained from patients with the following diseases: hepatitis C virus (23), hepatitis B virus (26), EBV (5), syphilis (33), tuberculosis (20), psoriasis (3), STD (62), pneumonia (12), toxoplasmosis (19), herpes simplex virus (5), cytomegalovirus infection (1), rubella virus (3), lymphadenopathy (2), Chlamydia infection (1), mycoplasmosis (3), autoimmune diseases (rheumatoid factors) (22), haemophilia (4), gammopathy (7) etc. Testing group also included 22701 serum samples from pregnant women (several times parous included). The average specificity of DIA-HIV ½ was 99.25 %: for pregnant women – 99.3%. The specificity of DIA-HIV ½ on testing of blood samples from 375 hospitalized patients was 99.89 %.

### Precision

The accuracy of DIA-HIV ½ kit was examined using the panel consisting of 3 HIV1-positive serum specimens (1 – high positive, 1 – medium positive, 1 – low positive), 3 HIV2-positive serum specimens (1 – high positive, 1 – medium positive, 1 – low positive), positive control (PC) and negative control (NC).

The specimens of the panel and controls were tested within INTRA-assay reproducibility by one performer using 3 test kit of the same lot number and the same equipment (36 repeat tests of the same samples) and within INTER- assay reproducibility by three performers during 3 days using 3 test kit of the same lot number and 3 different equipment sets (36 repeat tests of the same samples).

The coefficient of variation (CV) is in the range 5,2–7,5% (INTRA- assay reproducibility) and 8,4–11,9% (INTER- assay reproducibility) for OD values of six HIV positive sera of the panel.

### LIMITATIONS OF THE ASSAY

Accomplished studies demonstrated that DIA-HIV ½ is the high sensitive assay, however at the early stage of HIV-infection (period of “seroconversion window” – when only p24 antigen is present in serum or the level of antibodies is lower than a boundary of the method sensitivity) there is a possibility to obtain false negative results. This limitation of the assay was demonstrated in experimental conditions using some seroconversion panels. Besides, when testing in DIA-HIV ½ as highly sensitive immunoassay, a potential non-specific reactions must be taken into account. Therefore, the specificity of repeatable positive specimens must be verified using complementary test methods including Western Blot, the determination of p24 and Nucleic Acid Techniques. The influence of interfering endogenous factors in serum on the sensitivity and specificity of DIA-HIV ½ has been investigated. For this purpose native serum samples (or their mixtures) contained bilirubin, triglycerides, haemoglobins, total proteins and antibodies to E.coli in various titration series exceeding the normal level were involved:

Bilirubin	8.5-110 µM/L (norm: 8.5-20.5 µM/L)
Triglycerides	0.6-3.0 µM/L (norm: 0.5-1.6 µM/L)
Total proteins	67-90.8 mg/ml (norm: 65-85 mg/ml)
Hemoglobin	1.2-79 mg/ml
E.coli antibodies	¼ -1/128 (titre)

- Aspirate the contents of all wells and wash the plate with **washing solution** 8 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette into each well 100 µl of the **TMB substrate**.
- Cover the plate with an adhesive film and incubate at 18-25 °C for 30 minutes in the dark.
- 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 a dual wavelength microplate reader within 2 minutes after stopping the 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 – absorbance of the positive control

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

$\overline{PC}$  – mean absorbance of the positive control

- Calculate the mean absorbance of the negative control.

Test run is valid if  $\overline{NC}$  is not higher than 0.100.

Exclude any NC, which is higher than 0.100 or if it is than twice exceeded the  $\overline{NC}$ , and recalculate  $\overline{NC}$  of the remaining controls.

- Calculate  $\overline{PC}$ .

Test run is valid if  $\overline{PC}$  is not lower than 0.600.

- Calculate **Cut-off** value.

$$\text{Cut-off} = \overline{NC} + 0.100$$

- Determine the grey zone.

Grey zone is the zone with sample absorbance within the range

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

### Interpretation of the results

The result is considered as **nonreactive** if the specimen absorbance is below the grey zone.

The result is considered as **reactive** if the specimen absorbance is equal or greater than the cut-off.

Specimens with absorbance values within the grey zone range are considered **indeterminate** and should be retested with caution in duplicate.

Specimens shown an initially reactive or indeterminate result should be retested in two or more wells:

- specimens reactive in one or more wells are considered as reactive ones;
- specimens nonreactive in two or more wells are considered as nonreactive ones.

All repeatedly reactive results should be confirmed with an appropriate confirmatory methods.

## PERFORMANCE CHARACTERISTICS

### Sensitivity

- The sensitivity of DIA-HIV ½ was studied on testing of 1362 HIV-positive samples that included 873 HIV1, 132 HIV2, 367 samples containing antibodies to HIV in low titres (the commercial panels manufactured by BBI (USA), BCPI (USA) SFTS (France)), 85 samples of

- 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

- DIA-HIV ½ test kit is only intended for professional application and not to be subject to free sale in chemist's shop system. Performance of DIA-HIV ½ can be only realised in specially equipped diagnostic laboratories for ELISA that has appropriate permission (accreditation) of national state bodies/authorities. Special trained and skilled personnel is allowed to conduct assay performance.
- 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 and β-propiolacton. The absence of residual infectious capability of the positive control is confirmed by the validation. HOWEVER, ALL SAMPLES, CONTROLS AND MATERIALS USED FOR THE TEST PERFORMANCE MUST BE TREATED AND HANDLED AS BEING POTENTIALLY INFECTIOUS AND APPROPRIATE SAFETY PRECAUTIONS MUST BE TAKEN. 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.
- Any equipment directly in contact with specimens and reagents as well as the washing solution be considered as contaminated products and treated to good laboratory practice.
- 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 materials and products prior to utilization or discarding must be decontaminated following one of below mentioned methods:
  - 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 can be stored at 2-8°C but no longer than 72 hours. If necessary those specimens may be frozen (7 times freezing/thawing of samples do not influence on the performance characteristics of DIA-HIV ½) at temperature below -20°C. All specimens containing aggregates and visible suspended particles are to be clarified by centrifugation.

Specimens with sodium azide (more than 0.1 %), hyperlipidemiae (high lipaemic) or microbially contaminated serum or plasma samples cannot be used in ELISA procedure of DIA-HIV ½. Negative samples with high level of bilirubin (more than 100µM/L) may lead to unreliable results and should be considered as a risk of false positive results obtaining (see below Limitations of the assay).

There is no limitations in DIA-HIV ½ for testing of fresh serum samples.

DIA-HIV ½ is intended for testing of serum or plasma specimens obtained from blood preparations containing citrate, heparin and EDTA as anticoagulants. And others blood preparations such as purified globulins, albumins, blood factors etc. cannot be as samples for testing in DIA-HIV ½.

## ASSAY PROCEDURE

Reagents and specimens should reach a room temperature (18-25°C) before the assay performance and can remain at room temperature during testing. After use reagents should be returned to 2-8°C.

### Reagents preparation (for 8 wells)

#### Microplate

Open the pack 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 30 days at 2-8 °C after opening the pack.

#### 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. 4 ml of the concentrate is taken from the bottle and dissolved in 180 ml of the distilled or deionised water.

**Washing solution** is stable for 30 days at 2-8 °C.

#### Conjugate solution

Dilute 100 µl of the **Conjugate concentrate** with 1.0 ml of the **Conjugate diluent** (1:11) in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

**Conjugate solution** is stable for 30 days at 2-8°C.

#### TMB substrate

In order to prepare **TMB substrate**, dilute 0.5 ml of the **TMB solution** with 0.5 ml of the **Substrate buffer** 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 30 days 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.
- The temperature in room where performing analysis should be in the range 18-25°C.
- Reagents and samples should be at room temperature before testing begins. Return the reagents to 2-8 °C after use.
- 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.
- 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 a 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 buffer overflow from one well to the 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 by our technical services.

## Test procedure

- Fit the stripholder with required number of **strips**.
- Pipette 60 µl of the **conjugate solution** into each well.
- Distribute in the wells as follows:
  - wells A1, B1 : 30 µl of **positive control**.
  - wells C1, D1, E1 : 30 µl of **negative control**.
  - the rest wells : 30 µl of **samples**.

*Carefully repipette mixture in wells. (During repipetting solution turns its colour).*

- Cover the plate with an adhesive film and incubate at 37 °C for 90 minutes.