




OVERVIEW OF PROCEDURE

<ul style="list-style-type: none">• Dispense 100 µl both control specimens and investigated sera and 50 µl of conjugate solution in wells	
<ul style="list-style-type: none">• Incubate for 120 min at 42 °C• Wash 8 times with washing solution	
<ul style="list-style-type: none">• 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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DIA-HBV

**Enzyme immunoassay for the detection
of Hepatitis B surface antigen (HBsAg)**

96 tests

Product code: T-0207C

EXAMPLE FORM

INTENDED USE

Enzyme immunoassay kit is intended for the detection of the Hepatitis B virus surface antigen (HBsAg) in human serum or plasma.

INTRODUCTION

Today hepatitis B remains an actual objective of present-day sciences and health care in the whole world. It is stipulated by wide spreading of hepatitis B infection, a high level of disease incidence, quite often by a severe clinical course and also tendency to chronic liver affections – chronic hepatitis, cirrhosis and hepatocellular carcinoma.

The one of principal methods of specific diagnostics of hepatitis B is the detection of hepatitis B surface antigen (HBsAg). The revealing of HBsAg has important value for defining of an etiology of nature and differential diagnostics during viral hepatitis; HBsAg is marker that allows to evaluate the prevalence of hepatitis B at independent territories and among different investigated populations; to define groups of increased risk of hepatitis B infecting, etc. It is important to detect this marker during surveillance and testing of donor blood. Application of the whole testing of donor blood on HBsAg presence using ELISA methods with high performance characteristics has considerably decreased a number of incidents of posttransfusion hepatitis B.

PRINCIPLE OF PROCEDURE

DIA-HBV immunoenzyme test kit is based on principle of one-step “sandwich” ELISA. There is used a solid phase (microelisa stripplate) that coated with a monoclonal anti-HBs antibodies and conjugate (a monoclonal anti-HBs antibodies bound to a horseradish peroxidase).

When investigated specimen of human serum or plasma is placed into wells, HBsAg bind to specific antibodies on the solid phase, forming antigen-antibody complexes. Formed complexes are revealed with the help of specific immunoenzyme conjugate to HBsAg. Non-bound components are to be washed out.

The substrate buffer (hydrogen peroxide) and TMB solution is added to wells after washing non-bound components. Solution is coloured in case of presence of peroxidase conjugate in complexes.

To stop the colour reaction it is added stop-reagent and then determined the absorbance at 450/620 nm.

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 14 months.

KIT REAGENTS

For *in vitro* diagnostic use. Each kit contains:

No	Reagents	Presentation
1	Microplate strips 12 strips per plate each with 8 wells coated with monoclonal anti-HBs antibodies.	1 plate
2	Washing solution concentrate Phosphate buffer, containing Triton X100 and Tween 20.	2 bottles 2 × 25 ml
3	Positive control Human serum reactive for hepatitis B surface antigen (HBsAg). Inactivated by heating. Preservatives: 0.02 % 5-bromo-5-nitro-1, 3-dioxan, 0.04 % 2-methyl-4-isothiazolin-3-one.	2 vials 2 × 1.5 ml
4	Negative control Heating inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to HIV and hepatitis C virus (HCV). Preservatives: 0.02 % sodium azide.	3 vials 3 × 1.7 ml
5	Conjugate concentrate (11x) Monoclonal anti-HBs antibodies bound to a horseradish peroxidase (HRP). Preservatives: 0.1 % 2-methyl-4-isothiazolin-3-one.	1 vial 1 × 1.0 ml
6	Conjugate diluent Phosphate buffer, containing powdered milk. Preservatives: 0.02 % 2-methyl-4-isothiazolin-3-one.	1 bottle 1 × 9 ml
7	TMB solution Solution containing 0.03 % 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 8 ml
8	Substrate buffer Citrate-phosphate buffer, containing 0.016 % hydrogen peroxide.	1 bottle 1 × 8 ml
9	Stop-reagent 0.5M sulphuric acid solution.	1 bottle 1 × 15 ml
10	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);

Interpretation of the results

The result is considered as **nonreactive** one if the specimen absorbance is below the cut-off.

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

Specimens that show an initially reactive 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.

Performance characteristics of the test

Sensitivity

The sensitivity determining of DIA-HBV was carried by testing sera from patients with chronic hepatitis B and also sensitivity panels and samples with known content of HBsAg.

The sensitivity limit of DIA-HBV was evaluated using sensitivity panels manufactured by Boston Biomedica Inc. (USA), Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS, France), and samples with known content of HBsAg (Chemicon Int., USA)

Table 1

Sensitivity of DIA-HBV in panels of specimens HBsAg	
Panel or standard	Sensitivity DIA-HBV
AFSSAPS, subtypes adw2 and ayw3	0,3 ng/ml
PHA 806 (BBI), panel of specimens HBsAg, subtype ad	0,2 ng/ml
PHA 806 (BBI), panel of specimens, subtype ay	0,2 ng/ml
Chemicon, HBsAg, subtype ay	0,2 ng/ml
Chemicon, HBsAg, subtype ad	0,2 ng/ml

According to obtained results the sensitivity of DIA-HBV is within 0,2-0,3 ng/ml using sensitivity panels and specimens HBsAg subtypes ad and ay. When it was investigated 212 samples from persons with chronic hepatitis B form it was not obtained any false negative results.

Specificity

The specificity of DIA-HBV was evaluated using 8869 random donor samples and made 99,8 %. When it was investigated samples from hospitalize patients, pregnant women, samples positive for markers as CMV-infection, HSV-1, HSV-2, tuberculosis, rubella, syphilis, it was obtained not more than 0,3 % false positive results.

LIMITATION OF THE PROCEDURE

All highly sensitive immunoassay systems have a potential for non-specific reactions therefore the specificity of repeatable reactive specimens should be verified using appropriate test methods.

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 by our technical services.

Test procedure

- Fit the stripholder with required number of **strips**.
- Distribute in the wells as follows:
 - wells A1, B1: 100 µl of **positive control**.
 - wells C1, D1, E1: 100 µl of **negative control**.
 - the rest wells : 100 µl of **specimens**.
- Incubate the plate at 18-25 °C for 10 minutes.
- Pipette 50 µl of the **conjugate solution** into each well.
- Cover the plate with adhesive film and incubate at 42 °C for 120 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 8 times (according the section *Wash procedure*). 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 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

- Calculate the mean absorbance of the negative control.
All the values of NC should be lower than 0,100 optical units (OU).
If \overline{NC} is greater than 0,010 OU, exclude any NC that exceeds \overline{NC} more than twice.

If \overline{NC} is lower or equal to 0,010, all NC must lie within the interval from $-0,010$ to $+0,010$ of the NC average (from $\overline{NC} - 0,010$ to $\overline{NC} + 0,010$).
The aberrant value is to be eliminated, calculate again \overline{NC} with the other three values.
- The run is valid if PC is not lower than 0,600 OU.
- Calculate **Cut-off** value.

$$\text{Cut-off} = \overline{NC} + 0,06$$

- 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, 42±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 and chloroforme. 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, chromogen 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 at room temperature (18-25°C) before beginning the assay and can remain at room temperature during testing. After use return reagents to 2-8 °C after use.

Reagents preparation

Microplate strips

Open the pack and remove the plate. 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 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.

Dilute the washing solution concentrate 1:30 with distilled or deionised water (see chart below) shake intensively.

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

Number of wells	Stock washing solution (№1)	Distilled or deionised water
8	3 ml	90 ml
16	6 ml	180 ml
32	8 ml	240 ml
48	12 ml	360 ml
96	20 ml	600 ml

Conjugate solution

Dilute the 1:10 **conjugate** in **conjugate diluent** (see chart below) in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

Number of wells	Conjugate concentrate (11x)	Conjugate diluent
8	60 µl	0.6 ml
16	100 µl	1 ml
32	200 µl	2 ml
48	300 µl	3 ml
96	600 µl	6 ml

TMB substrate

To prepare **TMB substrate**, combine the required amount of **TMB solution** in a clean vial in equal parts with **substrate buffer** according to the number of wells being run (see chart below). Mix well. TMB substrate is to be colourless before use.

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

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.

Number of wells	TMB solution	Substrate buffer
8	0.5 ml	0.5 ml
16	1 ml	1 ml
32	2 ml	2 ml
48	3 ml	3 ml
96	6 ml	6 ml

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.
- 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 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