Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETERMINATION OF HBsAg IN HUMAN SERUM OR PLASMA

HBsAg EIA

REF **K009**

For 96 determinations

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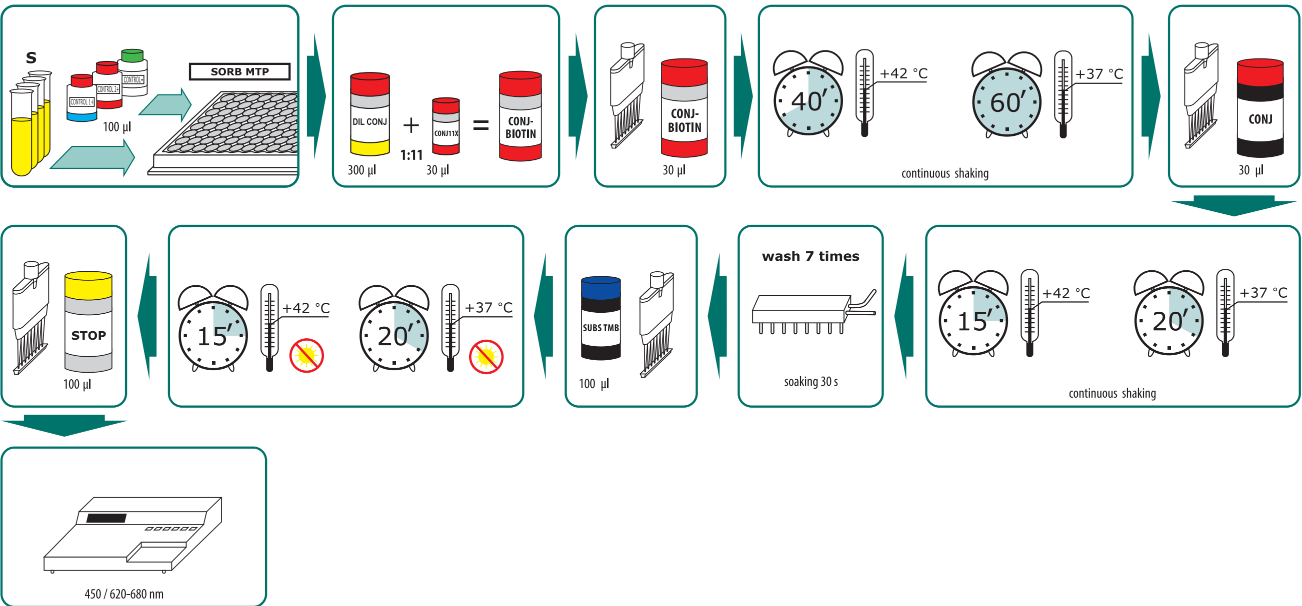
Test procedure



K009

CONTENT

1. INTENDED USE 2
2. SUMMARY AND EXPLANATION 2
3. PRINCIPLE OF THE TEST 2
4. WARNINGS AND PRECAUTIONS 3
5. KIT COMPONENTS 4
6. SPECIMEN COLLECTION AND STORAGE 6
7. TEST PROCEDURE 6
8. QUALITY CONTROL 8
9. CALCULATION OF RESULTS 8
10. EXPECTED VALUES 8
11. PERFORMANCE CHARACTERISTICS 8

Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETERMINATION OF HBsAg IN HUMAN SERUM OR PLASMA

1. INTENDED USE

A solid-phase enzyme immunoassay for the qualitative determination of HBsAg in serum or plasma.

This kit is designed for use with serum or plasma. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 45 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Hepatitis B infection is transmitted when virus particles come to blood (e.g., through transfusion of infected blood, use of infected syringes by drug takers, use of non-sterilized surgical instrument, cutting of personnel during surgical procedures on an infected patient), during sexual intercourse with infected partner, from infected mother to infant.

The main and the earliest marker of Hepatitis B infection is HBsAg in blood. HBsAg is a structural viral protein responsible for adsorption of viral particles on hepatocytes. Other markers which help to stage and classify the infection include HBeAg, IgG and IgM antibodies to HBcoreAg, IgG antibodies to HBsAg and HBeAg.

HBsAg appears in blood 3-5 weeks after infection. Then, during acute phase, anti-HBsAg antibodies appear and remove virus particles from blood. If 4-6 months after infection HBsAg disappears from blood, it is a good prognostic sign. If a patient remains HBsAg-positive, it may indicate chronic Hepatitis B.

HBsAg level in blood may vary significantly - from nanograms to hundreds of micrograms per ml.

ELISA is one of the most sensitive and widely used laboratory methods of HBsAg determination.

3. PRINCIPLE OF THE TEST

This test is based on sandwich enzyme immunoassay principle. Tested specimen and biotynilated monoclonal antibodies (Ab) to HBsAg (Conjugate A) are both placed into the microwells coated with polyclonal Ab to HBsAg. HBsAg from the specimen and conjugate A form a complex fixed on microwell surface by polyclonal Ab to HBsAg. This complex is detected by streptavidin-peroxidase conjugate (Conjugate B). After washing, the remaining enzymatic activity is detected by addition of chromogen-substrate mixture, stop-solution and photometry at 450 nm. Optical density in the microwells is directly related to HBsAg level in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for *in vitro* diagnostic use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5.0% H_2SO_4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Material Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.15. Material Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/ diluted components
1	SORB MTP	*HBsAg EIA strips, 8x12 wells*	polystyrene microwells coated with pAb to HBsAg	1	pcs	-	until exp.date
2	CONTROL -	Negative Control (3 ml)	preselected human serum with preservative - 0,01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; no coloration	1	pcs	colourless	2 months
3	CONTROL Ag+ CONTROL Ag++	Positive control sera (1 ml each*)	preselected human serum containing HBsAg standard grade, casein solution; preservative - 0,1% phenol; blue and red resp.	2	pcs	blue and red resp.	2 months
4	CONJ HRP	Conjugate B, 4 ml	aqueous solution of streptavidin coupled with horseradish peroxidase diluted on phosphate buffered solution, preservative - 0,01% Bronidox L, 0,01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; red dye	1	pcs	red	until exp.date
5	CONJ-BIOTIN-11X	concetrate of conjugate A, 0.5 ml	Biotynilated mAb to HBsAg in phosphate buffered saline; contains red dye	1	pcs	red	until exp.date
6	DIL CONJ	Buffer for dilution of concentrate of conjugate A, 5 ml	phosphate buffered saline and yellow dye	1	pcs	yellow	until exp.date
7	SUBS TMB	Substrate, 14 ml	tetramethylbenzidine (TMB) solution, ready to use.	1	pcs	colourless	until exp.date
8	BUF WASH 21X	Washing solution concentrate 21x, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate - until exp. date. Diluted washing solution - 45 days at 2-8 °C or 15 days at RT
9	STOP	Stop solution, 14 ml	5.0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp.date
10	N003	Plate sealing tape		2	pcs	-	N/A
11	K009I	Instruction HBsAg EIA		1	pcs	-	N/A
12	K009Q	QC data sheet HBsAg EIA		1	pcs	-	N/A

5.2. Equipment and material required but not provided

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µl;
- Microplate thermoshaker or a microplate shaker mounted in a 37 C incubator. Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 800-900/min;
- Calibrated microplate photometer with 450-620 nm wavelength and OD measuring range 0-3.0.

5.3. Storage and stability of the Kit

Store the whole kit at 2 to 8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

Specimens may be stored for up to 48 hours at +2...+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/ thawing should be avoided.

Important notice: biotin (vitamin B7) in a sample may cause incorrect results. Patients should avoid biotin-containing food (liver, eggs, cereals (esp., soy), pea, codfish, chicken, pistachios, milk products), drugs and/or supplements during three days before testing.

7. TEST PROCEDURE

7.1. Reagent preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18 to +25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 5 wells for the controls: CONTROL - (3 wells), CONTROL Ag+ and CONTROL Ag++ (1 well each) and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Pipet 100 µl of controls CONTROL -, CONTROL Ag+ CONTROL Ag++ and unknown samples into the wells.
3	Pipet 30 µl of working conjugate A solution into each well.
4	Incubate 60 minutes at 37°C and continuous shaking at 800-900 rpm OR for 40 minutes at 42 °C.
5	Dispense 30 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape.
6	Incubate 20 minutes at 37°C and continuous shaking at 800-900 rpm.
7	Prepare washing solution by 21x dilution of washing solution concentrate BUF WASH 21X by distilled water. Minimal quantity of washing solution should be 250 µl per well per wash. Wash the stripes 7 times with soaking during 30 sec.
8	Dispense 100 µl of SUBS TMB solution into the wells.
9	Incubate 20 minutes at +37 °C OR for 15 minutes at 42 °C.
10	Dispense 100 µl of STOP into the wells.
11	Measure OD (optical density) at 450 nm with 620 nm reference wavelength.
12	Set photometer blank on air.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specificati

8. QUALITY CONTROL

For the assay to be valid, the following requirements should be met:

1. OD450/620 for CONTROL Ag+ should be nlt 0.2 AU.
2. OD450/620 for CONTROL Ag++ should be nlt 1.0 AU.
3. OD450/620 for CONTROL- should not be more than 0.12 AU for all replicates.

9. CALCULATION OF RESULTS

1. Calculate the mean absorbance values (OD450/620) for CONTROL- in triplicates and each pair of samples.

2. Calculate the cut-off value: (mean OD450/620 for CONTROL-) + Coefficient A.

See Coefficient A value in the Quality control data sheet attached.

3. Calculate Positivity Index (PI) for each sample:

PI = mean OD450/620(sample) / Cut-off.

10. EXPECTED VALUES

If PI value is greater than 1.1, the result is POSITIVE.

If PI value is less than 0.9, the result is NEGATIVE.

If PI value is between 0.9 and 1.1, the result is EQUIVOCAL. Such samples should be retested. If the result is equivocal again, a new sample should be obtained 2-4 weeks later and tested again. If the result remains equivocal, the sample should be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity

Evaluation of a home-made panel of 1500 serum and plasma specimens confirmed HBsAg-negative gave 100% specificity.

11.2. Sensitivity

Analytical sensitivity.

The test demonstrated analytical sensitivity of nmt 0,01 IU/ml HBsAg when checked on a proficiency panel of HBsAg samples approved in Russia (manufactured by RMA Diagnostic Systems, Nizhny Novgorod, reg. cert. ФСР 2010/07216).

Diagnostic sensitivity.

When checked on a home-made panel of 1500 serum and plasma samples obtained from real patients and confirmed HBsAg-positive, the diagnostic sensitivity of the test was 100%.