DRG® HBsAg ELISA (EIA-5216)

As of 9 Mar. 2011 rm (Vers. 1.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

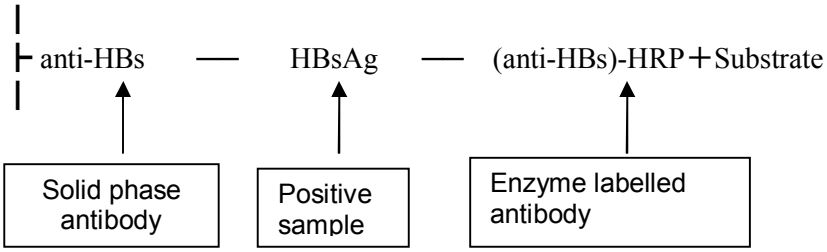
Not for use in diagnostic procedures.

1 INTENDED USE

HBsAg ELISA is for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

2 PRINCIPLE OF THE ASSAY

The test is an enzyme-immunoassay based on a 'sandwich' principle.



Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs (antibody to HBsAg), which constitutes the solid-phase antibody. The sample is incubated in such a well; HBsAg, if present in the sample, will bind to the solid-phase antibody. Subsequently guinea-pig anti-HBs, which has been labelled with the enzyme horseradish peroxidase (HRP), is added. With a positive reaction this labelled antibody is bound to any solid-phase antibody HBsAg complex previously formed. Incubation with enzyme substrate produces a blue color in the test-well, which turns yellow when the reaction is stopped with sulphuric acid. If the sample contains no HBsAg, the labelled antibody cannot be bound specifically and only a low background color develops.

3 PRESENTATION

Kits with test reagents for 96 tests (including test samples and controls) for easy recognition; the entire component is marked with the kit-specific yellow color.

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4 KITS CONTENTS

1. **Coated Microplate**: 1 plate (96 tests) Twelve 8-well strips per plate.
   Each microplate well is coated with plate monoclonal anti-HBs (mouse), and sealed in an aluminum bag containing a silica gel bag as desiccant.
2. **Conjugate:** 1 vial of 6.2 mL (HRP-labelled guinea-pig anti-HBs)
3. **Positive Control**: 1 vial of 1.0 mL
4. **Negative Control**: 1 vial of 1.0 mL
5. **Washing solution**: 1 bottle of 40 mL concentrated, which must be diluted 25-fold before use.
6. **Chromogen A**: 1 vial of 8.0 mL (contains hydro-peroxide).
7. **Chromogen B**: 1 vial of 8.0 mL (contains Tetramethylbenzidine (TMB).)
8. **Stopping solution**: 1 vial of 7.0 mL (2M H_2SO_4).
9. **Plate covers:** 2 pieces
10. **Instruction manual**: 1 copy

5 MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled water
2. Manual or automatic pipettors capable of delivering 20 µL, 50 µL, 100 µL and 1000 µL.
3. Disposable pipette tips.
4. Timer
5. Microplate mixer
6. Incubator (37 °C)
7. An automatic microplate washer (strongly recommended)
8. Microplate reader (equipped with a 450 nm and 630 nm filter)
9. Gloves

6 SAFETY PRECAUTIONS

The **positive control** contains HBsAg. Handle with gloves. The **negative control** is derived from human blood prepared only from donations which have been tested individually for HBsAg as well as for antibody to HIV by reliable methods and found to be negative. However, as no test method can offer complete assurance that infectious agents are absent all specimens of human origin should be considered potentially infectious and handled with gloves!

The **TMB solution** contains Dimethyl Sulphoxide, an irritant to skin and mucous membranes. (Avoid inhaling the vapors)!

Dispose of all specimens and materials used to perform the test as if they contained infectious agents.
Strip-holders and equipment should be disinfected after use e.g. with 2% glutaraldehyde, pH 7.5 - 8.0.

7 STORAGE AND STABILITY

If kept at 2 °C – 8 °C, all the test reagents are stable until the expiry date printed on the box.

When the aluminum bag has been opened, the unused strips can be safely stored at 2 °C – 8 °C in the sealable plastic pouch along with the silica gel placed inside.

After using a portion of the test reagents: conjugate, TMB, substrate, concentrated washing solution or controls, the remaining contents are stable until the expiry date, if kept at 2 °C – 8 °C sealed in the original vials.

8 SPECIMEN

Serum or plasma should be free of microbial contamination when tested.

Sodium azide (NaN_3) should not be added to the sample as a preservative.

Additives (other than gentamicin sulfate or proclin) and repeated freezing and thawing may give erroneous results.

Precipitates, clots and blood cells may cause an increased number of false positive results. Therefore insoluble material should be removed from all samples by centrifugation before testing.

9 REMARKS AND PRECAUTIONS

In one screening test-run do not combine strips, conjugate and controls from kits which have different lot numbers.

Do not perform the test in the presence of reactive vapors (e.g. from acids, alkalis or aldehydes) or dust, since the enzymatic activity of the conjugate may be affected.

All vials and bottles used for preparing the substrate solution must be cleaned thoroughly and finally rinsed with distilled water.

Strips may be used only once.

To avoid contamination, do not touch the edges of the wells with the pipette tips when adding sample, conjugate or substrate.

To avoid contamination, do not touch the top of the strips with your fingers.

All pipetting steps should be performed with the utmost care and accuracy.

Check for air-bubbles in the wells after all pipetting steps, if present, remove e.g. by gentle tapping.

Solutions containing TMB and/or peroxide should not come into contact with metals or metal- ions, since this may give rise to unwanted color formation

If the wells cannot be filled with substrate immediately after washing, the microplate may be placed face down on a wet absorbent tissue for no longer than 15 minutes.

10 DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test results. Operating instructions for the washing equipment should be followed.

Wash **five times** each plate. Blots dry the microplate by inverting the plate onto absorbent tissue, and striking a hard surface several times. If no automatic washer is available, washing can be performed manually as follows:

1. Aspirate thoroughly the liquid in the wells.
2. Then fill each well (0.3 mL) with diluted wash solution.

3. Allow the wash solution to remain in the wells for 5 seconds after filling.
4. Repeat these steps 5 times.
5. Complete the wash cycle by blotting the plate dry with absorbent tissue.

11 PREPARATIONS

1. Dilute the concentrated wash solution 1:25 with distilled water. The diluted wash solution must be at 20 °C to 25 °C when used.
2. Allow the test samples, controls, conjugate, diluted wash solution, substrate, aluminum bag containing the microplate and the vial containing TMB to come to ambient temperature before use.

12 PROCEDURE

1. Open the aluminum bag and take out the microplate with the required number of strips. The left strips are placed in the plastic pouch along with the silica gel bag, and sealed (see Storage and stability). During the test, the strips must stay in the microplate.
2. Pipette 50 µL of each specimen into the wells (leave 5 wells for controls and blank),
   pipette 50 µL of Positive Control into each of the two wells,
   and 50 µL of Negative Control into each of the two wells, and remain one blank, following the addition of the samples.
3. Pipette 50 µL of conjugate into each well (excluding the blank well).
4. Cover the strips with a plate sealer. Incubate at 37 °C for 60 minutes.
5. During incubation, dilute the concentrated washing solution 1:25.
6. Wash each well with the solution above five times and then blots dried by pressing plate onto absorbent tissue. (See Directions for washing).
7. Pipette 50 µL of Chromogen A into each well (including the blank well).
8. Pipette 50 µL of Chromogen B into each well (including the blank well).
9. Cover the plate with a fresh plate sealer. Incubate at 37 °C for 15 minutes in an incubator.
10. Stop the reaction by adding 50 µL of stopping solution to each well (including the blank well) and mix completely.
11. Photometric reading: Put the plate in the microplate reader and read (within10 minutes after step 10) the absorbance of the solution in the wells at 450 nm and 630 nm.

13 CALCULATION OF THE RESULTS OF THE SCREENING TEST

The judgement of results is based on the photometric reading data.

Abbreviations

N = the mean absorbance of the negative controls

P = the mean absorbance of the positive controls

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S = the absorbance of the test sample

Set the blank well you choose as blank, read the absorbance of the other wells.

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