DRG® HBsAb (EIA-5215)

As of 24 May 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.

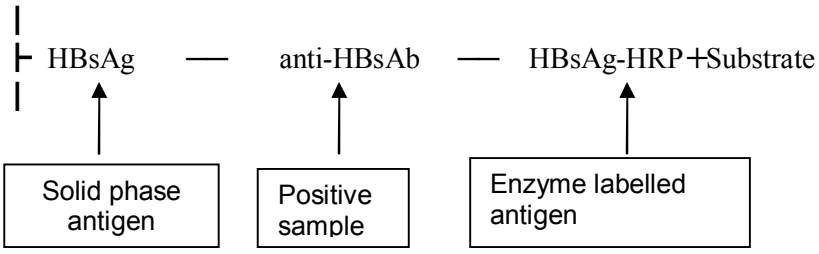
Not for use in diagnostic procedures.

1 INTENDED USE

The Anti-HBsAb is for the detection of antibody to hepatitis B surface antigen (anti-HBsAb) in human serum or plasma.
To investigate the humoral immunity against hepatitis B virus.

2 PRINCIPLE OF THE ASSAY

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



Polystyrene microtiter strip wells have been coated with hepatitis B surface antigen, which constitutes the solid-phase antigen. The test sample is incubated in such a well; anti-HBsAb, if present in the sample, will bind to the solid-phase antigen. In the mean time HBsAg, which has been labelled with the enzyme horseradish peroxidase (HRP), is added. With a positive reaction, solid-phase antigen/anti-HBsAb/HBsAg-HRP complex has been formed. Incubation with enzyme, hydroperoxide and TMB produce a blue color in the microwell, which turns yellow when the reaction is stopped with sulphuric acid. If the sample contains no anti-HBsAb, then the labelled antigen cannot be bound specifically and only a low background color develops.

3 PRESENTATION

Test reagents for 96 tests (including test specimens and controls).

4 KITS CONTENTS

1. **Coated Microplate**: 1 plate (96 tests), twelve 8-well strips per plate.
   Each microplate well is coated with hepatitis B surface antigen HbSAg and sealed in an aluminum bag containing a silica gel bag as desiccant.
2. **Conjugate**: 1 vial of 6.2 mL HRP-labelled HBsAg.

3. **Positive control**: 1 vial of 1.0 mL.
4. **Negative control**: 1 vial of 1.0 mL.
5. **Wash solution**: 1 bottle of 40 mL concentrate; which must be diluted 1:25 before use.
6. **Chromogen A**: 1 vial of 8 mL (contains hydroperoxide).
7. **Chromogen B**: 1 vial of 8 mL (containing Tetramethylbenzidine (TMB)).
8. **Stopping solution**: 1 vial of 7 mL (2M sulpuric acid).
9. **Plate covers**: 2 pieces.
10. **Instruction Manual**: 1copy.

5 MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled water.
2. Pipettes capable of delivering 20 µL, 100 µL, 1000 µL;
   the pipettes should not contain metal parts that can come into contact with the liquid.
3. Disposable pipettes tips.
4. Timer.
5. Incubator (37 °C).
6. Microplate washer (alternatively, washing can be performed manually, e.g. by using a repeating syringe delivering 0.3 mL volumes).
7. Microplate reader equipped with a 450 nm filter.
8. Absorbent tissue.
9. Gloves
10. Microplate mixer.

6 SAFETY PRECAUTIONS

The strips and conjugate contain HBsAg. The conjugate solvent and the controls are derived from human blood prepared only from donations which have been tested for HBsAg, and anti-HIV (I+II) by reliable methods 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 handled as if potentially infectious.

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

7 STORAGE AND STABILITY

If kept at 2 °C to 8 °C the test reagents, including strips, and positive and negative controls, are stable until the expiry date printed on the kit.

The aluminum bag should be brought to ambient temperature before opening to prevent condensation on the strips.

The unused strips should be placed in the plastic pouch along with the silica gel bag and stored at 2 °C to 8 °C.

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

Diluted wash solution is stable for 8 weeks at 2 °C to 8 °C.

8 SPECIMEN

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

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

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

9 REMARKS AND PRECAUTIONS

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

The incubator should have a relative humidity of at least 90%.

Ensure that the test samples and controls are homogeneous before use.

Strips may be used only once.

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

Ensure the microplate is sealed properly during incubation periods.

All pipetting steps should be performed with the utmost care and accuracy. To avoid false positive reactions caused by contamination do not touch the edges of the wells and the liquid in the wells with the pipette tips when adding sample or conjugate.

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

Proper washing after the incubation steps is essential to avoid incorrect reactions. Use the microplate washer, or carefully adhere to the directions for washing (see **Washing procedure**).

If the wells cannot be filled with substrate immediately after washing, the strip(s) may be placed upside down on a wet absorbent tissue for not longer than 15 minutes.

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

Ensure that the incubation time of the enzyme reaction in one test-run is the same for each well, by adding the sulphuric acid in the same sequence and at the same time intervals as the substrate solution.

Do not mix components from kits with different lot numbers.

10 WASHING PROCEDURE

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 unused strips can be stored in the plastic pouch provided that the silica gel bag is also included (See Storage and Stability). During the procedure the strips remain in the microplate. The strips can mark on the lower edge.
2. In the microplate leave 2 wells for negative control (50 µL each),
   2 wells for positive control (50 µL),
   and one well as a blank.
3. Add 50 µL of each specimen to be tested to the remaining wells.
4. Add 50 µL conjugate to each well (excluding the blank well).
5. Cover the microplate with a plate sealer and incubate for 30 minutes at 37 °C.
6. Wash each well for five times. (see **Washing procedure**)
7. Add 50 µL Chromogen A into each well (including blank well).
8. Add 50 µL Chromogen B into each well (including blank well).
9. Mix well and cover the plate with a fresh plate sealer. Incubate for 15 minutes at 37 °C.
10. Stop the reaction by adding 50 µL stopping solution to all the wells (including the blank well), and mix completely.
11. Microplate reading: Select the blank well, read the absorbance of the other wells (within 10 minutes after step 10) at 450 nm. If dual wavelength is used, the reference wavelength should be 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

S = the absorbance of the test sample

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

14 CALCULATION OF THE CUT OFF-VALUE

The cut-off value is 2.1 x N

If N is less than 0.05, then assume N = 0.05.

If N is greater than or equal 0.05, take the actual value for N

Test result:

A test is positive if S ≥ cut-off value

A test is negative if S < cut-off value

Checking of test-run validity

A test-run is only valid if N<0.1 and P>1.0.

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