DRG® HBcAb (EIA-5211)

As of 23 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.

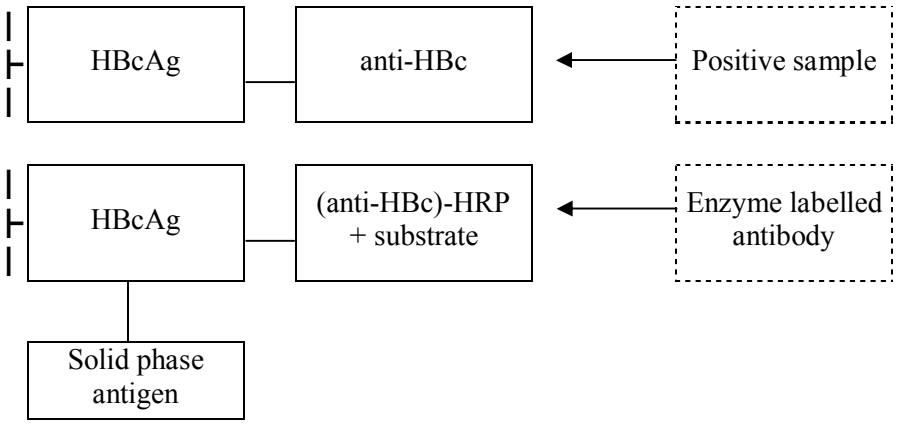
Not for use in diagnostic procedures.

1 INTENDED USE

The Anti-HBcAb ELISA is a kit for the detection of antibodies (IgG and IgM) to hepatitis B core antigen (anti-HBc) in human serum or plasma.

2 PRINCIPLE OF THE ASSAY

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



Polystyrene microtiter strip wells have been coated with hepatitis B core antigen, which constitutes the solid-phase antigen (HBcAg). The specimen and conjugate (mouse monoclonal anti-HBc labeled with HRP) are incubated in such a well at the same time. If the specimen contains anti-HBc, the coated HBcAg will be partially blocked, or completely blocked. The labeled antibody binds only to the the unblocked solid-phase antigen. 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 anti-HBc, only a reduced color develops compared with the negative control samples.

3 PRESENTATION

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

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4 KITS CONTENTS

1. **Coated Microplate:** 1 plate (96t ests), twelve 8-well strips per plate. Each microplate well is coated with HBcAg and sealed in an aluminum bag containing a silica gel bag as desiccant. (break apart)
2. **Conjugate**: 1 vial of 6.2 mL (HRP-labeled anti-HBc).
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 concentrated solution, which must be diluted 1:25 before use.
6. **Chromogen A:** 1 vial of 8 mL (containing hydro-peroxide).
7. **Chromogen B:** 1 vial of 8 mL (containing TMB).
8. **Stopping solution:** 1 vial of 7 mL (2M sulpuric acid).
9. **Plate covers:** 2 pieces.
10. **Instruction manual:** 1 copy.

5 MATERIALS REQUIRED BUT NOT PROVIDED

Distilled water and normal saline solution.
Pipettes capable of delivering 10 µL, 50 µL, 1000 µL etc.
Disposable pipettes tips.
Timer.
Incubator (37°C).
Microplate washer (alternatively, washing can be performed manually).
Microplate reader equipped with a 450 nm and 655 nm filter.
Absorbent tissue.
Gloves
Microplate mixer.

6 SAFETY PRECAUTIONS

Treat all serum and plasma specimens as if they are potentially infectious.
The HBcAg coated strips,conjugate,anti-HBc positive corotrol may be capable of transmitting hepatitis.
Do not use the kit after the expiration date.
Do not mix components from kits with different lot numbers.
Do not expose TMB reagents to strong light during storage or incubation.
Do not pipette by mouth.
Use only reagent grade quality, deionised or distilled water to dilute reagents.

Avoid contact of TMB and sulphuric acid with any oxidizing agent or metal. 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 test samples and controls are homogeneous before use.

Ensure the microplate is sealed properly during incubation periods.

Dispose of all specimens and materials used to perform the test as if they contained an infectious agent of viral hepatitis. The preferred method of decontamination is autoclaving for a minimum of one hour at 121.5°C. Decontaminate rubber gloves worn throughout the entire procedure before discarding. Disposable materials may be incinerated. Neutralized liquid wastes not containing acid, may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination.

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 box.

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 azide) and repeated freezing and thawing may give erroneous results.

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

9 NOTES ON TECHNIQUE

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.

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.

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 no 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. Blot 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 preformed 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 NECESSARY 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 be marked on the lower edge.
2. In the microplate leave 3 wells for negative control (50 µL each),
   1 well for positive control (50 µL),
   and one well as a blank.
3. Pipette 50 µL of each specimen to be tested to the remaining wells.
4. Pipette 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 five times.(see **washing procedure**)
7. Pipette 50 µL chromogen A into each well (including blank well).
8. Pipette 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

1. Abbreviations

S = the absorbance of the specimen

N = the mean absorbance of the negative controls

P = the mean absorbance of the positive controls

Calculate the absorbance for each specimen and control by subtracting the blank from each specimen and control value. If the blank correction is automatically performed by the microplate reader, omit this step.