

# HBcAb EIA Kit

*Enzyme-linked immunosorbent assay for the detection of antibody to HBcAg*

Catalog #:CT1005B

## INTRODUCTION

Anti-HBcAg antibody (HBcAb) EIA is a qualitative enzyme immunoassay for the detection of total antibody to core antigen of hepatitis B virus (HBc) in human serum or plasma. It is a competitive inhibition assay that features high specificity with a simple and fast procedure, using non-diluted serum or plasma specimen.

## SUMMARY AND PRINCIPLE OF THE TEST

Anti-HBc antibody test is a competitive enzyme immunoassay in which anti-HBc antibodies from specimens compete with a constant amount of Horseradish Peroxidase (HRP) conjugated anti-HBc antibody for a limited number of HBcAg coated on the microwells together with polyclonal antibodies against HBcAg as catcher.

The determination of anti-HBc antibody assay was described by Hoofnagle et al (1973). The test results can be used as an indicator to monitor the progress of hepatitis B viral infection. Anti-HBc is found in serum shortly after appearance of hepatitis B surface antigen (HBsAg) in acute hepatitis B and will persist after the disappearance of HBsAg and before the appearance of detectable antibody to HBsAg. Therefore, the determination of total antibody to HBcAg in serum or plasma can be of aid in the diagnosis of ongoing or previous hepatitis B viral infection.

Microtiter wells are coated with polyclonal antibodies against HBcAg and HBcAg. A serum specimen is added to the microtiter wells together with HRP conjugated anti-HBc antibody. After incubation, anti-HBc antibodies in specimen compete with constant amount of HRP-conjugated anti-HBc for limited amount of HBcAg in the wells. The unbound enzyme conjugates will be washed away and the chromogen substrate solution containing hydrogen peroxide is added to the wells for color development. Thus, the amount of HRP-conjugated anti-HBc bound to the well is inversely proportional to the concentration of anti-HBc antibody in the specimen. The absorbance of controls and specimens is determined by using EIA plate reader with wavelength set at 450 nm.

## REAGENTS

### *Materials provided with the kits:*

1. HBcAb Plate: One microplate with 96 wells coated with HBcAg.
2. Negative Control: One vial of 0.25 ml anti-HBc Negative Control.
3. Positive Control: One vial of 0.25 ml containing human anti-HBc antibody.
4. Enzyme Conjugate: 5.8 ml containing HRP-conjugated-anti-HBc for 96 tests.
5. Wash Buffer Concentrate (20X): 25ml for 96 tests. The buffer should be diluted 20 times with distilled water before use.
6. Substrate Solution A: 6.2 ml HRP Substrate for 96 tests.

7. Substrate Solution B: 5.8 ml TMB Chromagen Substrate for 96 tests.
8. Stop Solution: One bottle of 6.2 ml 2N Sulfuric Acid.

### *Materials required but not provided:*

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

## PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Must not use kit beyond the expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipet reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

## SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

## STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

## ASSAY PROCEDURES

1. Allow all reagents to reach room temperature before use.
2. Dispense 50 ul of Cut Off Reference, PC as well as Negative Control and 1:30 diluted specimen with washing buffer. into each well. Set one blank well as background control.
3. Add one drop (50 ul) of Enzyme Conjugate to each well. Mix them gently by swirling the microtiter plate on flat bench for 1 min.
4. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
5. Wash each well 4 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
6. Add one drop (50 ul) of Substrate Solution A to each well, then add one drop (50 ul) of Substrate Solution B to each well. Mix gently and incubate at 37°C for 15 min .

7. Add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Blank EIA plate reader with a blank control well and then read O.D. values of all samples at 450 nm.

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## INTERPRETATION OF RESULTS

### EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

The presence or absence of HBcAb is determined by comparing the absorbance value of the specimen to a cut-off value. The cut-off value is calculated from Negative and Positive Controls as explained in the calculations below.

Positive Result:

$$\frac{\text{OD value of Specimen}}{\text{Cut-Off Value}} \leq 1.0$$

Negative Result:

$$\frac{\text{OD value of Specimen}}{\text{Cut-Off Value}} > 1.0$$

Specimens with absorbance values within 10% of the cut-off value should be retested to confirm the initial test result.

### Calculation of Cut-off Value:

Cut-off value = OD value of NC x 0.3

## LIMITATIONS OF THE ASSAY

1. HBcAb EIA is limited to the detection of antibody against HBcAg in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of entire content of wells before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made only on the basis of a single test. A complete evaluation by a physician is needed for a final diagnosis.

## RELATED READING MATERIALS

1. Polesky H.F. and Olson C. The incidence and significance of antibody to Australia antigen in blood donors. Am. J. Clin. Pathol. 56: 129, (1971)
2. Blumberg B.S. et al., Australia antigen and hepatitis. New Eng. J. Med 283:349-354 (1970)
3. Hoofnagle J.H. et al., Antibody to Hepatitis B virus I core in man. Lancet 11:869-873, (1973)
4. Szmunes, W. et al. Antibody against the hepatitis type B core antigen. Am. J. Epidemiol.104: 256-262 (1976)

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FOR CLINICAL USE

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