

# HBeAb ELISA Kit

Enzyme-linked immunosorbent assay for the detection antibody against HBeAg in serum or plasma

Catalog #:CT1004B

## INTENDED USE

Anti-HBeAg antibody (HBeAb) EIA is a qualitative enzyme immunoassay for the detection of antibody to e antigen of hepatitis B virus (HBe) in human serum or plasma.

## SUMMARY AND PRINCIPLE OF THE TEST

The presence of antibody against hepatitis B viral e antigen is used as an indicator for (1) early HBs antigenemia before the peak of viral replication and (2) early convalescence when HBeAg has declined below detectable levels. It is also useful to confirm a seroconversion. The seroconversion from HBeAg positivity to anti-HBe positivity indicates a reduced level of infectious virus because virus replication has decreased.

Anti-HBe antibody test is a competitive enzyme immunoassay in which anti-HBe antibodies from specimens compete with a constant amount of Horseradish Peroxidase (HRP) conjugated anti-HBe antibody for a limited number of HBeAg in the neutralizing reagent added to the well. The microtiter well is coated with polyclonal antibodies against HBeAg as catcher for HBeAg. A serum specimen is added to the microtiter wells together with HRP conjugated anti-HBe-HBeAg complex. After incubation, anti-HBe antibodies in specimen, if present, compete with constant amount of HRP- conjugated anti-HBe for limited amount of HBeAg added into 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-HBe bound to the well is **inversely proportional** to the concentration of anti-HBe antibody in the specimen. The absorbance of controls and specimens is determined using EIA reader with wavelength set at 450 nm.

## REAGENTS

### Materials provided with the kits:

1. Microtiter Well coated with monoclonal anti-HBe: 96 tests
2. Negative Control: One vial of 0.25ml anti-HBe Negative Control.
3. Positive Control: One vial of 0.25ml anti-HBe Positive Control.
4. Enzyme Conjugate: 6 ml containing HRP-conjugated-anti-HBe-HBeAg complex for 96 tests.
5. Wash Buffer Concentrate (20 x): 25 ml for 96 tests. The buffer should be diluted 20 times with distilled water before use.
6. Substrate Solution A: 6 ml HRP Substrate for 96 tests.
7. Substrate Solution B: 6 ml TMB Chromagen Substrate for 96 tests.
8. Stop Solution: One bottle of 6 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 PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Dispense one drop (50 ul) of Cut Off Reference, Positive Control as well as Negative Control in duplicate into respective wells. Set one black well as background control, and 50ul of serum or plasma samples into respective wells.
3. Add one drop (50 ul) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
4. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
5. Wash each well 5 times by filling each well with diluted wash buffer, then invert the plate vigorously to get all water out and block the rim of each well 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. Read O.D. at 450 nm with an EIA reader.

## INTERPRETATION OF RESULTS

### Result Interpretation:

The presence or absence of HBeAb 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: Specimen whose absorbance value is equal to or less than the cut-off value is to be considered reactive and positive for HBeAb.

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

Negative Result: Specimens with absorbance value greater than the cut-off value is considered negative for HBeAb.

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

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:

$$\text{Cut-off value} = \text{OD value of NC} \times 0.5$$

If the negative control OD value is higher than 1.5, reported as 1.5. If it is lower than 1.5, reported as actual value.

## LIMITATIONS OF THE ASSAY:

1. HBeAb EIA is limited to the detection of antibody against HBeAg 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 completely before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.

## RELATED READING MATERIALS:











1. Magnus L.O., et al. New antigen-antibody system. Clinical significance in long term carriers of hepatitis B surface antigen. J. Am. Med. Asso. 231: 356-359 (1975)
2. Aldershvile J. et al., Hepatitis B e antigen and antibody measured by radioimmunoassay in acute hepatitis B surface antigen positive hepatitis. J. Infect. Dis. 141: 293-298, (1980)

3. Mushahwar, I.K. et al., Prevalence of hepatitis B e antigen and its antibody as detected by radioimmunoassays. *J.Med.Virol.* 2:77-87(1978)

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	<i>In vitro</i> diagnostic device		Lot code
	Consult instructions for use		Catalogue number
	Keep dry		Contains sufficient for <n> tests
	Temperature limitation		Manufacturer
	Use by		Do not use if package damaged