

# HBeAg EIA Kit

**Enzyme Linked Immunosorbent Assay for the Detection Of Hbe Antigen**

Catalog #: CT1003B

## INTENDED USE

HBeAg EIA is a qualitative enzyme immunoassay for the detection of HBeAg in human serum or plasma.

## SUMMARY AND PRINCIPLE OF THE TEST

The Hepatitis B e antigen is found to be related to Hepatitis B viral infection. HBeAg is usually found in the early phase of Hepatitis B viral infection. The titer of HBeAg rises rapidly during the period of virus replication. The presence of HBeAg correlates with increasing numbers of infectious viruses (Dane particles) and the presence of viral specific DNA polymerase in serum. During the HBeAg positive stage, hepatitis B patients are at increasing risk of transmitting the virus to their contacts. Persistent presence of HBeAg in the hepatitis B virus carrier is often associated with chronic active hepatitis.

The HBeAg EIA is a sandwich immunoassay, which employs monoclonal and polyclonal antibodies specific for HBeAg. Microtiter wells are coated with monoclonal antibodies specific for HBeAg. A serum specimen is added to the antibody coated microtiter wells together with enzyme conjugated polyclonal antibodies. HBeAg, if present, will form an antibody-HBeAg-antibody-enzyme complex. The plate is then washed to remove unbound materials. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop in proportion to the amount of HBeAg present in the specimen. The enzyme substrate reaction can be stopped and the result is visualized by the naked eye or obtained by EIA plate reader for absorbance at wavelength of 450 nm.

## REAGENTS

### *Materials provided with the kits:*

1. Microtiter Well coated with monoclonal anti-HBe: 96 tests in one bag.
2. Negative Control: One vial of 0.25ml HBeAg Negative Control.
3. Positive Control: One vial of 0.25ml HBeAg Positive Control.
4. Enzyme Conjugate: 5.8 ml containing HRP-conjugated-anti-HBe 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: 6.2 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 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 4 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

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

Positive: P/N value is equal to or greater than 2.1

Negative: P/N value is less than 2.1

$$P/N \text{ value} = \frac{\text{OD value of specimen}}{\text{Average OD value of Negative Control}}$$

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

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#### LIMITATIONS OF THE ASSAY

1. HBeAg EIA is limited to the detection and semi-quantitative of HBeAg in serum or plasma. 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.
2. 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. Magnius L.O., Lindholm, A. Lundin, P and Iwarson, S.A., New antigen-antibody system. Clinical significance in long-term carriers of hepatitis B surface antigen. J.Am.Med.Assoc.231:356
2. 359 (1975).
3. Aldershvile, J. et al., Hepatitis B e antigen and anti-body measured by radioimmunoassay in acute hepatitis B surface antigen positive hepatitis. J. Infect. Dis. 141: 293-298, 1980.
4. 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.
5. Lin, C.M. et al., Hepatitis B e antigen and its correlation with other serological markers in chimpanzees. Inf. Imm. 24: 352-356, 1979.
6. Lander, J.J., et al. Frequency of antibody to hepatitis associated antigen as measured by a new radioimmunoassay technique. J. Immunol. 106:1166-1171(1971).

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