The Anti-HBe ELISA is designed for the qualitative determination of antibodies to hepatitis B e antigen (anti-HBe) concentration in human serum or plasma specimens.

**INTENDED USE**

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**INTRODUCTION**

Hepatitis B is a disease caused by viral infection. The route of infection can be improper needle puncture, blood transfusion or even by taking contaminated food or water. The presence of antibody against hepatitis B viral e antigen is used as an indicator for early HBs antigenemia before the peak of viral replication and early convalescence when HBeAg has declined below detectable levels. It is also useful to confirm a sero-conversion. The sero-conversion from HBeAg positive to anti-HBe positive indicates a reduced level of infectious virus because virus replication has decreased.

**BIOLOGICAL PRINCIPLE OF THE PROCEDURE**

Anti-HBe test is a competitive enzyme immunoassay in which monoclonal antibody (MAb) to hepatitis B e antigen (HBeAg) is pre-coated on microwells, then HBeAg is attached to the solid phase through the pre-coated anti-HBe. Anti-HBe in specimens competes with a constant amount of hors eradish peroxidase (HRP) conjugated anti-HBe MAb for the limited number of HBeAg on the wells. The unbound materials will then be washed away and the chromogen substrate will remain stable for at least 2 months, or until the labeled expiration date, whichever is earlier, provided it is stored as prescribed above.

**MATERIALS PROVIDED**

1. Coated Microwells (refer to principle): 1 plate, 96 wells.
2. Enzyme Conjugate Reagent: HRP labeled anti-HBe MAb in stabilizing buffer (1 vial, 7.5ml).
3. Negative Control: Heat inactivated healthy human sera diluted in buffer (1 vial, 0.8ml).
4. Positive Control: Human sera containing purified and inactivated anti-HBe and diluted by normal human serum. (1 vial, 0.8ml).
5. Stock Wash Solution: PBS-Tween (1 bottle, 30ml, 20X)
6. Substrate Solution: Hydrogen peroxide (1 vial, 7.5ml)
7. Chromogen Solution: Tetramethyl benzidine (TMB) (1 vial, 7.5ml)
8. Stop Solution: 1.0M H2SO4 (1 vial, 7.5ml)

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Micropipettes and multi-channel micropipettes of appropriate volume
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Incubator
6. Disposable reagent troughs
7. Instrumentation

- Automated microplate strip washer
- Microplate reader
- Fully automated microplate processor

**STORAGE OF TEST KIT AND INSTRUMENTATION**

1. Unopened test kits should be stored at 2 – 8°C upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. All opened components will remain stable for at least 2 months, or until the labeled expiration date, whichever is earlier, provided it is stored as prescribed above.
3. Return other unused components to 2 – 8°C. Use within the expiry date.

**SPECIMEN COLLECTION AND PREPARATION**

1. Plasma samples may be used but serum is the recommended sample type for this assay.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation. 4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2 – 8°C. Specimens going to be stored or transported for more than 48 hours must be stored frozen (- 20°C or lower). Avoid multiple freeze-thaw cycles. After thawing, ensure specimens are thoroughly mixed and brought to room temperature before being assayed.
6. Do not add sodium azide into the specimen as a preservative.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Reserve 1 well for blank, add 50 μl of Negative Control to each of the 3 consecutive wells, 50 μl of Positive Control to each of the next 2 wells and 50 μl of each sample to each of the remaining wells. Then add 50 μl of Enzyme Conjugate Reagent into each well except the blank well.
3. Incubate the plate at 37°C for 30 minutes. Do not pipette by mouth. Use protective clothing and disposable gloves.
4. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.
5. Please read the instruction for use carefully prior to use, and carry out the test strictly according to time and temperature indicated on the instructions. 6. Read the result within 20 minutes after terminating the test.

**REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18 – 25°C) prior to use, place at room temperature for at least 30 minutes, place at room temperature for at least 30 minutes.
2. Adjust the incubator to 37°C.
3. Add 50ml of Wash Solution Concentrate to 1000ml of distilled water, mix and add with a magnetic stirrer. The Wash Solution is stable at room temperature for two months.

**PRECAUTIONS AND WARNINGS**

1. For in vitro diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. Avoid any skin contact with all reagents. Stop Solution contains H2SO4, in case of contact, wash those thoroughly with water.
4. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.
5. Please read the instruction for use carefully prior to use, and carry out the test strictly according to time and temperature indicated on the instructions. 6. Read the result within 20 minutes after terminating the test.

**IMPORTANT NOTES**

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. Do not reuse the plate covers.
4. It is recommended that no more than 32 wells be used for each assay run if manual pipette is used. A full plate of 96 wells may be used if automated pipette is available. Dispensing of all specimens and controls should be completed within 5 minutes.
5. Replace caps on reagents immediately. Do not switch caps.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Reserve 1 well for blank, add 50 μl of Negative Control to each of the 3 consecutive wells, 50 μl of Positive Control to each of the next 2 wells and 50 μl of each sample to each of the remaining wells. Then add 50 μl of Enzyme Conjugate Reagent into each well except the blank well.
3. Incubate the plate at 37°C for 30 minutes.
5. Wash the microplate 6 times with the Wash Solution prepared, by hand or using an automated microplate strip washer. After washing is completed, invert the microplate and tap out any residual Wash Solution onto absorbent paper.
6. Dispense 50µL of Substrate solution into each well.
7. Dispense 50µL of Chromogen solution into each well. Gently mix horizontally for 15 seconds.
8. Incubate the plate at 37°C in the dark for 10 minutes without shaking.
9. Add 50 µL of Stop Solution to each well. Mix gently.
10. Immediately after mixing, read the absorbance of each well at 450 nm in a microplate reader using 620 – 630 nm as the reference wavelength. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

**INTERPRETATION OF**

1. Test is valid if
Mean absorbance of Negative Controls is greater than 0.8.
Mean absorbance of Positive Controls is less than 0.1.
2. Calculation of cut-off value
Cut-off value = (mean absorbance of Negative Controls + mean absorbance of Positive Controls)/2
3. Interpretation of results
Any specimen with an absorbance greater than or equal to the cut-off value is considered negative.
Any Specimen with an absorbance less than the cut-off value is considered positive.

**PERFORMANCE CHARACTERISTICS**

1. Sensitivity
The sensitivity of the test is in concordance with that of other commercial tests.
2. Specificity
No evident cross reactions were observed with HAV, HCV and HIV markers. The assay was not interfered by RF factors, specimens containing high bilirubin concentrations, grossly hemolytic and lipemic specimens.

**LIMITATIONS**

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results, in particular, correct sample and reagent dispensing, along with careful washing and timing of incubation steps is essential for an accurate, reproducible detection of antibodies to HBcAg.
2. Use fresh plasma samples, or samples frozen and thawed only once. Samples degradation as well as multiple freeze-thaw cycles may cause erroneous results. Do not use heat inactivated samples.

3. As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the results of a single test. A complete evaluation by a physician is needed for a final diagnosis.
4. This assay can only test plasma or serum samples. The reliability to test other body fluids is not confirmed.

**QUALITY**

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.