**PRINCIPLE OF THE TEST**

This kit is based on solid phase, one step incubation competitive principle ELISA method. Anti-HBc if present in the sample, compete with monoclonal anti-HBc conjugated to horseradish peroxidase (HRP-Conjugate) for a fixed amount of purified HBcAg pre-coated in the wells. When no anti-HBc presents in the sample, the HRP-conjugated anti-HBc will be bound with the antigens inside the wells and any unbound HRP-Conjugate is removed during washing. TMB A and B solutions are added into the wells and during incubation, the colorless TMB are hydrolyzed by the bound HRP-Conjugate antibody to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. No or low color developing suggests for presence of antibodies to HBcAg in the sample.

**INTENDED USE**

This kit is an enzyme-linked immunosorbent assay for qualitative detection of antibodies to hepatitis B virus core antigen (anti-HBc) in human serum or plasma. It is intended for use in clinical laboratories for diagnosis and management of patients related to infection with hepatitis B virus.

**MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multi-channel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37°C incubator.

**STORAGE**

Unopened Kit: Store at 2 - 8°C. Do not use past kit expiration date.

Opened/Reconstituted Reagents: TMB Solution A; TMB Solution B; TMB Stop Solution; Wash Buffer; HRP-conjugate antibody

The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.

Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip seal. May be stored for up to 1 month at 2 - 8°C.

**PRECAUTIONS**

1. The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. When mixing or reconstituting protein solutions, always avoid foaming.
3. Do not mix or substitute reagents with those from other lots or sources.
4. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
**SPECIMEN TREATMENT**

Centrifuge the serum, plasma or cell culture supernatant samples for 10 minutes at 1,000×g. Remove particulates and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

**ELISA PROTOCOL**

1. Allow the reagents and samples to reach room temperature for at least 15-30 minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash Buffer 1 to 20 with distilled or deionized water. Only use clean vessels to dilute the buffer.

2. Set the strips needed in strip-holder and number sufficient number of wells including three Negative controls (e.g. B1, C1, D1) two Positive controls (e.g. E1, F1) and one Blank (e.g. A1). Neither samples nor HRP-Conjugate should be added into the Blank well. If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

3. Add 50 µl specimen, 50 µl Negative control and 50 µl Positive control into their respective wells. Note: Use a separate disposal pipette tip for each specimen, Negative, Positive Control as to avoid cross-contamination.

4. Add 50 µl of antibody HRP-Conjugate antibody to each well except the blank. Mix by tapping the plate gently.

5. Cover the plate with the plate cover and incubate the plate for 30 minutes at 37°C. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during incubation.

6. Remove and discard the plate cover. Wash each well 5 times with diluted washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.

7. Dispense 50 µl of TMB Solution A and 50 µl TMB Solution B into each well including the Blank, and mix by tapping the plate gently. Incubate the plate at 37°C for 10 minutes avoiding light. The enzymatic reaction between the TMB solutions and the HRP-Conjugate antibody produces blue color in Negative control and negative sample wells.

8. Remove and discard the plate cover. Using a multichannel pipette or manually, add 50 µl Stop solution into each well and mix gently. Intensive yellow color develops in Negative control and negative sample wells.

9. Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction.)

**INTERPRETATION OF RESULTS AND QUALITY CONTROL**

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample’s optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Calculation of Cut-off value:

   Cut-off value (C.O.) = *Nc × 0.5

   *Nc = the mean absorbance value for three negative controls.

**Example:**

1. Calculation of Nc:

   Well No     | B1 | C1 | D1 |
   Negative controls OD value | 1.720 | 1.715 | 1.717 |

   Nc = 1.717

2. Calculation of Cut-off value: Cut-off (C.O.) = 1.717 × 0.5 = 0.856

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:

   The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

   1) The OD value of the Negative control must be equal to or greater than 0.500 at 450/630nm, or at 450nm after blanking.
   2) The OD value of the Positive control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

   (S = the individual optical density (OD) of each specimen)

   **Negative Results (S/C.O. > 1):** Samples giving an absorbance greater than the Cut-off value are considered negative, which indicates that no antibodies to HBV core antigen have been detected using this anti-HBc ELISA kit. This result should not be used alone to establish the infection state.
Positive Results (S/C.O. ≤1): Samples giving absorbance less than, or equal to the Cut-off value are initially reactive for this assay, which indicates that antibodies to HBV core antigen have probably been detected with this anti-HBc ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for anti-HBc. A positive result with anti-HBc detection indicates acute HBV infection. Determination of anti-HBc is useful for screening of blood donors and in serological monitoring during follow-up of chronic HBV carriers. However, any positive result should not be used alone to establish the infection state.

Borderline (S/CO = 0.9-1.1): Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline samples and retesting is recommended. Repeatedly reactive samples could be considered positive for anti-HBc.

**LIMITATIONS**

1. Non-repeatable positive results may occur due to the general biological and biochemical characteristics of ELISA assays. The test is designed to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
3. The prevalence of the marker will affect the assay’s predictive values.
4. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

**INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS**

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such cases, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones, or contact our technical support for further assistance.
2. If after mixing of the TMB Solution A and B into the wells, the color of the mixture turns blue within a few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

**REFERENCES**