**Antibody to Hepatitis B Virus Core Antigen Elisa Kit**

*Cat. No.:DEIA1001
Pkg.Size:96T*

### 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.

### General Description

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver diseases, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of a number of serological markers expressed during three phases (incubation, acute and convalescent) of the infection. Now several diagnostic tests are used for screening, clinical diagnosis and management of the disease. Hepatitis B “core” antigen (HBcAg) is a major component of the viral structure. HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregation of the core particles; the antigen contains at least one immunological determinant. Antibodies to HBcAg (anti-HBc total antibody, and IgM) appear shortly after the appearance of HBsAg and persist for life both in persons who have recovered from a hepatitis B infection and in those who develop HBsAg-carrier status but in rare cases, a HBV infection can also run its course without the appearance of immunologically detectable anti-HBc (usually in immunosuppressed patients). Anti-HBc is a marker of acute, chronic or resolved HBV infection and screening for anti-HBc provides with information on the prevalence of the disease in different groups. In clinical diagnosis, the detection of anti-HBc is an important diagnostic marker and together with other hepatitis B test permits the diagnosis and management of the disease. In the absence of other hepatitis B markers (HBsAg-negative persons), anti-HBc may be the only indication of an existing hepatitis B viral infection.

### 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. Chromogen A and B solutions are added into the wells and during incubation, the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate 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.

### Reagents And Materials Provided

**MICROWELL PLATE:** 1plate

Blank microwell strips fixed on a white strip holder. The plate is sealed in aluminum pouch with desiccant. 8×12/12×8-well strips per plate. Each well contains purified HBcAg.

The microwell strips can be broken to be used separately.

Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2-8°C.

**NEGATIVE CONTROL:** 1vial

Yellowish liquid filled in a vial with green screw cap.
1 ml per vial.
Protein-stabilized buffer tested non-reactive for anti-HBc. Preservatives: 0.1% Proclin 300.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.
POSITIVE CONTROL: 1vial
Red-colored liquid filled in a vial with red screw cap.
1 ml per vial.
Purified anti-HBc diluted in protein stabilized buffer containing preservatives: 0.1% Proclin 300.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.
HRP-CONJUGATE REAGENT: 1vial
Red-colored liquid filled in a white vial with red screw cap.
6.5 ml per vial.
Horseradish peroxidase-conjugated anti-HBc.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.
STOCK WASH BUFFER: 1bottle
DILUTE BEFORE USE
Colorless liquid filled in a clear bottle with white screw cap.
30ml per bottle.
PH 7.4, 20 × PBS (Contains Tween-20 as a detergent).
The concentrate must be diluted 1 to 20 with distilled or deionized water before use. Once diluted, stable for one week at room temperature or for two weeks when stored at 2-8°C.
CHROMOGEN SOLUTION A: 1vial
Colorless liquid filled in a white vial with green screw cap.
7ml per vial.
Urea peroxide solution.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.
CHROMOGEN SOLUTION B: 1vial
Colorless liquid filled in a black vial with black screw cap.
7ml per vial.
TMB solution (Tetramethyl benzidine dissolved in citric acid).
Ready to use as supplied.
PLASTIC SEALABLE BAG: 1unit
For enclosing the strips not in use.
CARDBOARD PLATE COVER: 1sheet
To cover the plates during incubation, and prevent the well from evaporation or contamination.
PACKAGE INSERTS: 1copy

**Materials Required But Not Supplied**

Freshly distilled or deionized water.
Disposable gloves and timer.
Appropriate waste containers for potentially contaminated materials.
Disposable V-shaped troughs.
Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
Absorbent tissue or clean towel.
Dry incubator or water bath, 37±0.5℃.
Microshaker for dissolving and mixing conjugate with samples.
Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
Microwell aspiration/wash system.

Storage

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 ℃; do not freeze. To assure maximum performance of this anti-HBc ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Specimen Collection And Handling

Sample Collection: Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

Assay Steps

Step1 Reagents preparation: Allow the reagents and samples to reach room temperature (18-30℃) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37℃ until crystals dissolve. Dilute the stock Wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 Numbering Wells: 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.

Step3 Adding Sample and HRP-Conjugate: Add 50μl of Positive control, Negative control, and Specimen into their respective wells. Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination. Add 50μl of HRP-Conjugate to each well except into the Blank and mix by tapping the plate gently.

Step4 Incubating: Cover the plate with the plate cover and incubate for 60minutes at 37℃. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step5 Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remaining liquids.

Step6 Coloring: Dispense 50μl of Chromogen A and 50μl Chromogen B solution into each well including the Blank. Incubate the plate at 37℃ for 15minutes, avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate will produce blue color in Negative control and anti-HBc negative sample wells.

Step7 Stopping Reaction: Using a multichannel pipette or manually, add 50μl Stop Solution into each well and mix gently. Intensive yellow color develops in Negative control and anti-HBc negative sample wells.

Step8 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 5minutes after stopping the reaction).
Quality Control

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 Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450nm.
2. The OD value of the Negative control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Positive control must be less than 0.100 at 450/630nm or at 450nm after blanking.

Calculation

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 (C.O.) = *Nc × 0.5
   *Nc = the mean absorbance value for three negative controls.
   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.

Interpretation of Results

S = the individual absorbance (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 e antigen have been detected using this anti-HBe 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 e antigen have probably been detected with this anti-HBe ELISA kit. Any reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for anti-HBe. This result should not be used alone to establish the infection.

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

Sensitivity

Analytical Endpoint Sensitivity: The sensitivity of the assay has been calculated by means of the reference standards provided from the Reference Laboratory for Immunology Product under the Ministry of Health. The assay shows sensitivity at the Cut-off of 1NCU (National Current Unit, MOH).

The clinical sensitivity of this anti-HBc ELISA kit have been calculated by a panel of samples obtained from 975 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. This panel included samples from acute, chronic and recovered hepatitis B patients. Licensed anti-HBc ELISA test was used as a confirmatory assay. The evaluation results are given below. Results obtained in individual laboratories may differ.
Specificity

The clinical specificity of this assay has been determined by a panel of samples obtained from 1683 healthy blood donors and 145 undiagnosed hospitalized patients. The repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of the specificity.

Analytical Specificity:
1. No cross reactivity observed with samples from patients infected with HAV, HCV HIV, CMV, and TP.
2. No interference from rheumatoid factors up to 2000U/ml observed during clinical testing.
3. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.
4. Frozen specimens have been tested to check for interferences due to collection and storage.

Precautions

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.
1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. CAUTION - CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well’s bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450 nm or at 450 nm with reference at 630 nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1 hour at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2 M H2SO4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. Proclin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkanics etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

**Limitations**

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of the ELISA method. 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. Antibodies may be also undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
3. 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.
4. The prevalence of the marker will affect the assay’s predictive values.
5. This is a qualitative assay and the results cannot be use to measure antibodies concentrations.
6. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.

**REFERENCES**