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User's Manual

Human anti-Hepatitis B Surface Antibody, anti-HBsAb ELISA Kit

REF **DEIA002**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

Principles of Testing

For detection of anti-HBs, this kit uses antigen "sandwich" ELISA method where polystyrene microwell strips are pre-coated with recombinant HBsAg. Patient's serum or plasma sample is added to the microwells together with a second HBsAg conjugated to Horseradish Peroxidase (HRP-Conjugate). In case of presence of anti-HBs in the sample, the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and during incubation, the specific immunocomplex formed is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-Conjugates, Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-antibody-antigen(HRP) "sandwich" complex, 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. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HBs remain colorless.

Reagents And Materials Provided

1. Human HBsAg Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with purified HBsAg;
2. Negative Control: 1ml, 1 vial;
3. Positive Control: 1ml, 1 vial;
4. HRP-Conjugate Antibody: 6.5ml, 1 vial;
5. TMB Solution A: 7ml, 1 vial;
6. TMB Solution B: 7ml, 1 vial;
7. TMB Stop Solution: 7ml, 1 vial;
8. Wash Buffer (20×): 30ml, 1 vial;
9. Microtiter plate sealers: 1 sheet;
10. Plastic Sealable Bag: 1 unit.

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 multichannel.
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 zipseal. May be stored for up to 1 month at 2-8°C.

Specimen Collection And Preparation

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.

Assay Procedure

1. Reagents preparation: Allow the reagents and samples to reach room temperature (18-30°C) for at lest 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 stock Wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer.
2. Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including three Negative control (e.g. B1, C1, D1), two Positive control (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. Adding Sample and HRP-Conjugate: Add 50ul 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 50l HRP-Conjugate to each well except the Blank and mix by tapping the plate gently.
4. Incubating: Cover the plate with the plate cover and incubate for 60 minutes at 37°C. 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.
5. Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash 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 it plate to remove any remaining liquids.
6. Coloring: Dispense 50ul of Chromogen A and 50ul Chromogen B solution into each well including the Blank, and mix by tapping the plate gently. Incubate the plate at 37°C for 15minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and in anti-HBs positive sample wells.
7. Stopping Reaction: Using a multichannel pipette or manually, add 50ul Stop Solution into each well. Intensive yellow color develops in Positive control and anti-HBs Positive sample wells.

8. 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

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 Positive control must be equal to or greater than 0.500 at 450/630 nm, or at 450 nm after blanking.

2) The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450 nm after blanking.

3)Interpretations of the results: (S = the individual optical density (OD) of each specimen)

Negative Results (S/C.O.<1): samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no antibodies to hepatitis B virus surface antigen have probably not been detected with this kit. Therefore, there are no serological indications for past infection and the individual is not immune against infection with HBV.

Positive Results (S/C.O.≥1): samples giving an absorbance greater than or equal to the Cut-off value are initially reactive, which indicates that antibodies to HBV surfaces antigen have been detected using this anti-HBs ELISA kit. Retesting in duplicates of any reactive samples is recommended. Repeatedly reactive samples could be considered positive for anti-HBs. Elevated concentrations of anti-HBs are indication for recovery and immunity to HBV.

Borderline: Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these samples in duplicates is recommended to confirm the results. Repeatedly positive samples can be considered positive for antibodies to HBsAg.

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.

Calculation of Cut-off value

Cut-off value (C.O.) = *Nc × 2.1

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

Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05.

Example:
1. Calculation of Nc:

Well No	B1	C1	D1
Negative controls OD value	0.02	0.012	0.016

Nc=0.016(the Nc value is lower than 0.05 so take it as 0.05)
2. Calculation of Cut-off value: Cut-off (C.O.)= 0.05 x 2.1= 0.105

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.

Sensitivity

Human Anti-HBsAb ELISA Kit

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.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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 indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, 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 few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

Limitations

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is design 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. 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.
3. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
4. 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.
5. The prevalence of the marker will affect the assay's predictive values.
6. 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.
7. This is a qualitative assay and the results cannot be use to measure antigens concentrations.

References

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