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Human Hepatitis B surface Antibody IgG Quantitative Eilsa Kit

Product Number: HBA01

Shipping and Storage

1. 2-8℃, stored away from light and moisture
2. Validity period: 6 months

Component

Component	96T	48T
Microporous enzyme-linked immunosorbent assay (ELISA) plate	12 holes×8 strips	12 holes×4 strips
negative control	0.5mL	0.5mL
positive control	0.5mL	0.5mL
Detect antibody HRP	10mL	5mL
20× washing buffer solution	25mL	15mL
Sample diluent	6mL	3mL
Substrate A	6mL	3mL
Substrate B	6mL	3mL
Stop solution	6mL	3mL
Microplate Sealers	2 sheets	2 sheets

Description

The reagent kit adopts indirect enzyme-linked immunosorbent assay (ELISA). Add the sample and HRP labeled detection antibody to the pre coated micropores of hepatitis B virus surface antigen, incubate and thoroughly wash. Using substrate TMB for color development, TMB is converted to blue under the catalysis of peroxidase and to the final yellow under the action of acid. Measure the absorbance (OD value) at a wavelength of 450nm using an enzyme-linked immunosorbent assay (ELISA) reader, and compare it with the CUTOFF value to determine the presence or absence of hepatitis B virus surface IgG antibodies (HBs IgG) in the specimen.

Specimen collection and storage

1. Serum: Use test tubes without pyrogen and endotoxin, avoid any cell irritation during the operation, collect blood, centrifuge at 3000 rpm for 10 minutes, and quickly and carefully separate serum and red blood cells.
2. Plasma: EDTA, Citrate or heparin anticoagulant. Centrifuge at 3000 rpm for 30 minutes and collect the supernatant.
3. Cell supernatant: Centrifuge at 3000 rpm for 10 minutes to remove particles and polymers.
4. Tissue homogenate: Crush the tissue by adding an appropriate amount of physiological saline. Centrifuge at 3000 rpm for 10 minutes and collect the supernatant.

Materials required but not supplied

1. ELISA reader (450nm)
2. High precision sampler and nozzle: 0.5-10μL, 2-20μL, 20-200μL, 200-1000μL.
3. 37℃ constant temperature box.

Note

1. The reagent kit should be stored at 2-8℃ and equilibrated at room temperature for 20 minutes before use. The concentrated washing solution taken out of the refrigerator will have crystals, which is a normal phenomenon. Heating in a water bath will

completely dissolve the crystals before use.

2. The Flat noodles not used in the experiment should be immediately put back into the self sealing bag, sealed (low-temperature drying) and stored.
3. Please dilute the preprocessed sample appropriately with sample diluent according to the operating steps to achieve the best detection effect of the reagent kit.
4. Strictly follow the time, liquid dosage, and sequence indicated in the instructions for incubation operation.
5. Shake all liquid components thoroughly before use.

Reagent Preparation

20×dilution of washing buffer: Dilute distilled water at a ratio of 1:20, which means adding 19 parts of distilled water to 1 part of 20×washing buffer.

Washing method

Manual board washing: Shake off the liquid in the holes, fill each hole with washing solution, let it stand for 1 minute, shake off the liquid in the holes, pat dry on absorbent paper, and wash the board 5 times in this way.

Automatic washing machine: Inject 350μL of washing solution into each well, soak for 1 minute, and wash the plate 5 times.

Protocol

1. Take out the required Flat noodles from the aluminum foil bag after 20 min of room temperature balance, and seal the remaining Flat noodles with a self sealing bag and put it back at 4 ℃.
2. Set up negative and positive control wells and sample wells, and add 50μL of negative control and 50μL of positive control to each well;
3. Add 10μL of the test sample to the well, followed by 40μL of sample diluent;
4. Subsequently, 100μL of horseradish peroxidase (HRP) labeled detection antigen was added to each well of the negative, positive control, and sample wells. The reaction wells were sealed with a sealing membrane and incubated at 37 ℃ in a water bath or constant temperature incubator for 60 minutes.
5. Discard the liquid, pat dry on absorbent paper, fill each well with detergent, let it stand for 1 minute, shake off the detergent, pat dry on absorbent paper, repeat washing the board 5 times (or use a board washing machine).
6. Add 50μL of substrate A and B to each well, and incubate at 37℃ in the dark for 15 minutes.
7. Add 50μL of termination solution to each well and measure the OD value of each well at a wavelength of 450nm within 15 minutes.

Result judgment

1. Experimental effectiveness: The average OD value of the positive control wells is ≥1.00; The average OD value of negative control wells is ≤ 0.15.
2. Cut off calculation: Cut off=Mean value of negative control wells+0.15.
3. Negative judgment: If the OD value of the sample is less than the critical value (Cut off), the sample is negative.
4. Positive judgment: If the OD value of the sample is greater than the critical value (Cut off), the sample is positive.

Test kit performance

1. Accuracy: The average OD value of positive control wells is ≥ 1.00; The average OD value of the negative control wells is ≤ 0.15, indicating the validity of the test results.
2. Specificity: Does not cross react with other soluble structural analogues.
3. Repeatability: The coefficient of variation within and between plates is less than 15%.