For scientific research use only and not for clinical testing.

优品生物
UpingBio

Human hepatitis B virus surface antigen (HBsAg) quantitative detection kit (ELISA)

manual

【product name】

Generic name: Human hepatitis B virus surface antigen (HBsAg) quantitative detection kit (ELISA)

[Packaging specifications]

48 servings/box, 96 servings/box

【expected usage】

For scientific research use only, quantitatively detect the concentration of human hepatitis B virus surface antigen (HBsAg) in serum, plasma, and cell culture supernatant.

[Testing Principle]

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-human hepatitis B virus surface antibody (solid-phase antibody), add human hepatitis B virus surface antigen (HBsAg) calibrator and sample to be tested, and then add HRP-labeled anti-human Hepatitis B virus surface antibody (enzyme-labeled antibody), after incubation and sufficient washing, removes unbound components, and forms a sandwich complex of solid-phase antibody-antigen-enzyme-labeled antibody on the solid surface of the microplate. Add substrates A and B. Under the catalysis of HRP, the substrate produces a blue

product. Under the action of stop solution (2M sulfuric acid), it is finally converted into yellow. The absorbance (OD value) is measured on a 450nm microplate reader. The absorbance (OD value) is positively correlated with the concentration of human hepatitis B virus surface antigen (HBsAg) in the sample to be tested. By fitting the calibrator curve, the concentration of human hepatitis B virus surface antigen (HBsAg) in the sample can be calculated.

【Main components】

Main ingredients

Components	quantity	Main ingredients
Calibrator	0.3ml/tube*6 tubes	6 concentration standards formulated for antigen
coated microplate	96T/48T	Pre-coated solid phase antibodies
HRP labeled antibodies	6mL	HRP-labeled detection antibodies
sample diluent	6mL	Buffer
Substrate solution A	6mL	hydrogen peroxide working fluid
Substrate solution B	6mL	TMB working fluid
20×concentrated washing liquid	25mL	PBS with 0.15% Tween20
stop solution	6mL	acidic solution
manual	1 serving	--
Ziplock bag	1	--
self-adhesive	2 tablets	--

The concentrations of calibrators are: 8, 4, 2, 1, 0.5, 0 ng/ml. The calibrator has been tested and the results show that it is negative for HIV1, HIV2 and HCV antibodies. Since there is no test method that can completely guarantee the absence of these substances, this product must be handled as potentially infectious and the handling process should follow general safety measures. .

Materials and consumables required but not provided 1. Microplate reader 2. Precision pipette and disposable tips 3. Distilled water 4. Washing bottle or automatic plate washer 5. 37°C water bath or incubator 6. 500ml measuring cylinder 7 , Powder-free disposable latex gloves 8. Quality control products

[Storage conditions and validity period]

1. Store at 2-8°C. Do not freeze. The validity period is 6 months.

2. After opening and use, place the coated microplate into a ziplock bag with desiccant, seal the ziplock bag, and return all reagents to the 2-8°C refrigerator.

3. After opening, store according to the recommended conditions. The calibrator, coated microplate and HRP-labeled antibody are valid for 14 days. Other components are stable within the validity period indicated on the label.

[Applicable instruments]

Semi-automatic microplate reader, such as Thermo MK3,
or domestic microplate reader.

【Sample requirements】

Sample type and collection

The following lists only general guidelines for sample collection. During all sample collection processes, sodium azide must not be used as a preservative. 1. Cell culture supernatant: Centrifuge at 4000 rpm for 20 minutes to remove cell particles and polymers. Store the supernatant below -20°C to avoid repeated freezing and thawing.

2. Serum: Use test tubes that do not contain pyrogens and endotoxins. Avoid any cell stimulation during the operation. Centrifuge at 4000 rpm for 20 minutes. Carefully separate the serum and store it below - 20°C. Avoid repeated freezing and thawing.

3. Plasma: Heparin, EDTA, or sodium citrate as anticoagulant. Centrifuge at 4000 rpm for 20 minutes to collect the supernatant. Store the plasma below -20°C to avoid repeated freezing and thawing.

Sample preservation and stability

Samples can be stored at 2-8°C for 72 hours, or at -20°C for 6 months. After the sample is collected, it is not necessary to test it all at once. Please pack and freeze it according to the one-time use to avoid repeated freezing and thawing. Thaw it at room temperature when using it to ensure that the sample is evenly and fully thawed.

[Test method] Reagent preparation 1. Before use, all components must be
rewarmed for at least 30 minutes to ensure sufficient rewarming to room
temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Dilute concentrated washing liquid and distilled water at 1:20, that is, add 1 part of concentrated washing liquid to 19 parts of distilled water.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

Operating procedures 1. Return all reagents and components to room temperature first. It is recommended to make duplicate holes for standards, quality control materials and samples.

2. Prepare working solutions for various components of the kit according to the method described in the previous reagent preparation.

3. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

4. Set up standard wells, sample wells and blank wells. Add 50 μL of standards of different concentrations to each of the standard wells. Add 50 μL of the sample to be tested to the sample wells. Do not add any to the blank wells.

5. In addition to the blank wells, add 50 μL of horseradish peroxidase-labeled detection antibody to the standard wells and sample wells. 6. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 60 minutes.

7. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, and pat dry on absorbent paper.

8. Repeat this 4 times (wash the plate 5 times in total). If you use an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 20 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

9. Mix substrates A and B thoroughly at a volume of 1:1, and add 100 μL of substrate mixture to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

10. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450nm microplate reader.

[Interpretation of test results]

After the detection is completed, use the concentration of the standard substance as the ordinate and the corresponding absorbance (OD value) as the abscissa. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance of the sample (OD value), use the equation to calculate the concentration value of the sample.

If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.

[Limitations of inspection methods]

1. For scientific research use only and not for clinical diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then measure again. 6. Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

[Product performance indicators]

1. Physical properties

Each liquid component of the kit should be clear and transparent, without sediment or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

2. Linear dose response curve

Calibrator dose response curve correlation coefficient r value, greater than or equal to 0.9900.

3. Precision

Intra-batch precision: Three groups of known high, medium and low concentration samples were evaluated twenty times in the same plate. The intra-batch coefficient of variation CV% is less than 10%.

Inter-batch precision: Three groups of known high, medium and low concentration samples were evaluated twenty times in different sections. The inter-batch variation coefficient CV% is less than 15%.

4. Sensitivity

The lowest detectable dose was less than 0.1 ng/ml.

5. Recovery rate

Three groups of known high, medium and low concentration samples were evaluated five times for recovery rates, and the recovery rates ranged from 85% to 115%.

6. Specificity

This kit recognizes native and recombinant human hepatitis B virus surface antigen (HBsAg) and has no crossover with structural analogs.

7. Stability

Store at 2℃-8℃, valid for 6 months.

【Precautions】

Biosecurity

1. Testing must comply with laboratory management regulations, and cross-contamination must be strictly prevented. All samples, wash solutions and various wastes must be disposed of as infectious agents.

2. The liquid component of the kit contains proclin-300 preservative, which may cause skin allergic reactions. Avoid inhaling smoke and contact with skin.

3. The substrate liquid is irritating to the skin, eyes and upper respiratory tract. Avoid inhaling the smoke. Wear protective gloves and wash hands thoroughly after completing the experiment.

Technical Tips 1. When mixing the protein solution, avoid foaming.

2. When adding calibrators and samples, the pipette tip must be replaced for each calibrator concentration and sample, and common components should be cantilevered to avoid cross-contamination.

3. Appropriate incubation time and sufficient washing steps are necessary to ensure the accuracy of experimental results.

4. The substrate solution is a colorless liquid. If it turns blue during storage, it means that the substrate solution has expired and must not be used. 5. The order of adding the stop solution is consistent with the order of adding the substrate solution. After adding the stop solution, the blue substrate product will instantly turn to yellow.

6. During the experiment, the remaining slats should be immediately put back into the ziplock bag and sealed (low temperature drying) for storage.

7. Shake all liquid components thoroughly before use, and perform incubation operations in strict accordance with the time, sample volume, and sample addition order indicated in the instructions.

waste disposal

All used or unused reagents, and all contaminated disposable materials, should follow the handling procedures for infectious or potentially infectious products. Each laboratory is responsible for waste and contaminated materials according to the type and hazard level of its experiment. All waste and dirt must be treated in strict accordance with relevant regulations.