仅供科研使用，不得用于临床检验

For scientific research use only, not for clinical testing.

人乙型肝炎病毒表面抗原（HBsAg）定性检测试剂盒（ELISA）说明书

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

[Product name] Generic name: Human hepatitis B virus surface antigen (HBsAg) qualitative detection kit (ELISA) English name: Huamn hepatitis B virus surface antigen (HBsAg) ELISA KIT

[Packaging specifications]
96 servings/box

【expected usage】

For scientific research use only, Qualitative detection of human hepatitis B virus (HBsAg).

[Testing Principle]

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). To the microwells pre-coated with human hepatitis B virus surface antigen (HBsAg) capture antibody, add specimens, negative and positive controls in sequence, and then add another strain of HRP-labeled anti-human hepatitis B virus surface antigen (HBsAg).) Antibody (enzyme-labeled antibody), after incubation and sufficient washing, unbound components are removed, and a sandwich complex of solid-phase antibody-antigen-enzyme-labeled antibody is formed 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 depth of the color is related to the human hepatitis B virus surface antigen (HBsAg) in the sample.) is positively correlated. Use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm to determine negative and positive.

【Main components】

Main ingredients 组份 数量 主要成分

Components	quantity	Main ingredients
negative control	0.3ml/tube	--
positive control	0.3ml/tube	--
coated microplate	96T/48T	Pre-coated solid phase antigen
HRP labeled antibodies	10mL/5mL	HRP-labeled detection antibodies
Substrate solution A	6mL/3mL	carbamide peroxide working solution
Substrate solution B	6mL/3mL	TMB working fluid
sample diluent	6mL/3mL	--
20×concentrated washing liquid	30mL/15mL	PBS with 0.15% Tween20
stop solution	6mL/3mL	0.2mol dilute sulfuric acid
manual	1 serving	--
Ziplock bag	1	--
self-adhesive	2 tablets	--

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℃. 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 4000rpm for 20 minutes to remove cell particles and Below 20℃, 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 for 20 minutes at 4000 rpm to take 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 the sample 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 the concentrated washing liquid and distilled water 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 negative control wells, positive control wells and sample wells. Add 50 μL of control substance to each of the negative and positive control wells. Add 50 μL of the sample to be tested to the sample well. Do not add any to the blank well.

5. In addition to the blank wells, add 100 μL of horseradish peroxidase-labeled detection antigen to the negative control wells, positive control 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 plate washer operating procedure. 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 microplate reader.

[Interpretation of test results] 1. Negative control OD value: less than 0.2. 2. Positive control OD value: greater than 0.8.

3. Positive judgment (Cut-Off value): If the negative control OD value is +0.35, and the sample OD value is greater than the threshold, it is judged as positive, otherwise, it is negative.

[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. 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%.

3. Specificity

This kit recognizes native and recombinant human hepatitis B virus surface antigen (HBsAg) without crossover with structural analogs.

4. Stability

Store at 2℃-8℃, valid for 6 months.

[Notes]

Biosafety

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, 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. Dispose of waste and dirt in strict accordance with relevant regulations.