优品生物
UpingBio

For scientific research use only, not for clinical diagnosis

人乙型肝炎病毒核心(HBcAg）定量检测试剂(ELISA）

Quantitative detection of human hepatitis B virus core antigen in serum, plasma, and cell culture supernatant

(HBcAg) concentration.

Before using the kit, you must read this instruction manual carefully.

实验原理

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 core antigen (HBcAg) antibody (solid-phase antibody), add the human hepatitis B virus core antigen (HBcAg) calibrator and the sample to be tested, and then add HRP The labeled anti-human hepatitis B virus core antigen (HBcAg) antibody (enzyme-labeled antibody) is incubated and fully washed to remove unbound components and form a solid-phase antibody-antigen-enzyme label on the solid surface of the microplate. Antibody sandwich complexes. Add substrates A and B. Under the catalysis of HRP, the substrate produces a blue product. Under the action of the stop solution (2M sulfuric acid), it is finally converted into yellow. The absorbance (OD value) is measured on the microplate reader at a wavelength of 450nm. The absorbance (OD value) is positively correlated with the concentration of human hepatitis B virus core antigen (HBcAg) in the sample to be tested. By fitting the calibrator curve, the concentration of human hepatitis B virus core antigen (HBcAg) in the sample can be calculated.

试剂盒限制性

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.

技术提示

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. Common components should be added with a cantilever. Avoid cross contamination.
3. Appropriate incubation time and sufficient washing steps are necessary to ensure the accuracy of experimental results.
4. When using an automatic plate washer, adding a 30-second soaking step can improve detection accuracy.

5. 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. 6. 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.

color.

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

8. Shake all liquid components thoroughly before use, and warm them in strict accordance with the time, sample amount and sample addition order indicated in the instructions. education operation.

试剂盒组分与保存

Store unopened kits at 2-8 degrees and do not use expired kits.

组分	数量	主要成分	开封后储存
Calibrator	0.3ml/tube	--	2-8℃14 days
coated microplate	96T/48T	Pre-coated solid phase antibodies	2-8℃14 days
HRP labeled antibodies	10mL	HRP-labeled detection antibodies	2-8℃180 days
sample diluent	6mL	--	2-8℃180 days
Substrate solution A	6mL	0.01% hydrogen peroxide	2-8℃180 days
Substrate solution B	6mL	0.1%TMB	2-8℃180 days
stop solution	6mL	acidic solution	2-8℃180 days
20×concentrated washing liquid	25mL	0.05%Tween20	2-8℃180 days
manual	1 serving	--	--
Ziplock bag	1	--	--
self-adhesive	2 tablets	--	--

The concentrations of calibrators are: 8, 4, 2, 1, 0.5, 0.25 ng/mL.

其他用品

1. Microplate reader (450nm)

2. Precision pipette and

disposable tips

3. Distilled water 4. Bottle

washing or automatic

plate washing machine

5. 37℃ water bath or thermostat

6. 500ml measuring cylinder

生物安全

1. Testing must comply with laboratory management regulations and strictly prevent cross-contamination. All samples, discarded solutions and various waste

Objects should be handled as infectious agents.

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

Contact with skin.

3. The substrate liquid is irritating to the skin, eyes and upper respiratory tract. Avoid inhaling the smoke.

4. Wear protective gloves and wash hands thoroughly after completing the experiment.

样品的采集和储存

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative.

1,**细胞培养上清：** Centrifuge at 4000 rpm for 20 minutes to remove cell particles and polymers, and store the supernatant at - 20°C. Below, avoid repeated freezing and thawing.

Use pyrogen- and endotoxin-free test tubes, avoid any cell stimulation during operation, and centrifuge at 4000 rpm. 2.**血清：**

Incubate for 20 minutes, carefully separate the serum, and store it below -20°C to avoid repeated freezing and thawing.

3.**血浆：** Heparin, EDTA, or sodium citrate as anticoagulants. Centrifuge at 4000rpm for 20 minutes to take the supernatant. Plasma should be stored below -20°C and avoid repeated freezing and thawing.

After the sample is collected, it cannot be tested at one time. Please pack and freeze it according to the amount used once to avoid repeated freezing and thawing. Thaw it at room temperature when using it to ensure that the sample is evenly and fully thawed.

4.**组织匀浆：** Rinse the tissue with pre-cooled PBS (0.01 M, pH=7.4) to remove residual blood, weigh and cut into pieces.

Mix the minced tissue with the corresponding volume of PBS (generally according to a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9 mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add it in PBS Add protease inhibitor) into a glass homogenizer and grind thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or repeatedly frozen and thawed. Finally, centrifuge the homogenate at 5000 × g for 5-10 minutes, and take the supernatant for detection.

5. Cell extraction solution: Wash the adherent cells gently with cold PBS, then digest them with trypsin, and collect the cells after centrifugation at 1000×g for 5 minutes; suspended cells can be collected by centrifugation directly. Collected cells were washed three times with cold PBS. Add 150-200μL PBS per 1×106 cells to resuspend and disrupt the cells by repeated freezing and thawing (if the content is very low, the volume of PBS can be reduced). Centrifuge the extract at 1500 × g for 10 minutes, and take the supernatant for detection.

6. Other biological fluids: Centrifuge at 1000×g for 20 minutes to remove impurities and cell debris. Take the supernatant for testing.

试剂准备

1. Before use, all components must be rewarmed for at least 120 minutes to ensure full 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. Concentrated washing liquid and distilled water are diluted 1:20, that is, 1 part of concentrated washing liquid is added 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.

操作程序

All reagents and components should be returned to room temperature first. It is recommended to perform duplicate wells for standards, quality control materials and samples.

1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.

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

Set up standard wells, 0 value wells, blank wells and sample wells. Add 50 μL of standards of different concentrations to each of the standard wells. Add 50 μL of sample diluent to the 0 value well. Do not add it to the blank well. Add 50 μL of the sample to be tested to the sample well.

3. In addition to the blank wells, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to the standard wells, 0 value wells and sample wells.

4. Cover the reaction plate with sealing film and incubate in a 37℃ water bath or incubator in the dark for 60 minutes.

5. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing liquid, let it stand for 20 seconds, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If using an automatic plate washer, please wash the plate according to the plate washer operating procedure. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

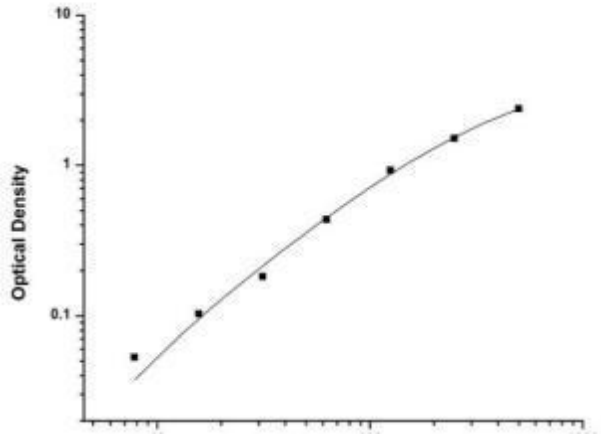
6. Mix substrates A and B thoroughly at a volume of 1:1, and add 100 μL of substrate mixture to all wells. Cover the reverse with sealing film Place the plate on the plate and incubate in a 37℃ water bath or incubator in the dark for 15 minutes.

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

结果计算

1. Use the standard concentration as the abscissa (6 standard wells, plus 1 0 value well, a total of 7 concentration points), and the corresponding absorbance (OD value) as the ordinate, use computer software, and use a four-parameter Logistic Curve fitting (4-pl), create a standard curve equation, and use the absorbance (OD value) of the sample to calculate the concentration value of the sample.

2. 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.



(Schematic diagram, for reference only)

试剂盒性能指标

1、物理性能

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、剂量反应曲线线性

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

3、精密度

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、灵敏度

The lowest detectable dose is less than 0.1 ng/mL.

5、回收率

Three sets of known high, medium and low concentration samples were evaluated for recovery five times in the same plate, with recovery rates ranging from 85% to 115%.

6、特异性

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

7、稳定性

Store at 2℃-8℃, valid for 6 months.

8、 检测范围

0.25 ng/mL－8 ng/mL.