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User's Manual

Human HBcAg ELISA Kit

REF **DEIA-ZH0011-H**

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

HBV Core Antigen ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the HBV core protein. The kit has detection sensitivity limit of 1 ng /mL HBcAg. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HBV samples.

General Description

Hepatitis B is an infection of the liver caused by the hepatitis B virus. HBV is transmitted by exposure to infectious blood or body fluids (e.g. saliva, semen, urine); forms of transmission include unprotected sexual activity, blood transfusion, mother-to-infant transmission, or consuming contaminated food/water. The acute illness causes liver inflammation, vomiting and jaundice, while chronic HBV infection often leads to liver cirrhosis and cancer. Roughly one third of the world's population have been infected with hepatitis B virus. 5-10% of adults and 90% of babies who have been infected will have the virus for the rest of their lives. The infection is preventable by vaccination. Diagnosis of chronic hepatitis B virus (HBV) infection has long been based on HBV serology and measurement of hepatocytic enzymes. With the development of therapies for chronic HBV infection, including interferon and lamivudine, quantitative detection of HBV has been used increasingly as the most important marker for monitoring HBV replication activity, disease progression, and assessing antiviral treatment.

Principles of Testing

An anti-HBVcAg monoclonal coating antibody is adsorbed onto a microtiter plate. HBV core antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a FITC-conjugated mouse anti-HBVcAg antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-HBVcAg. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of HBV core antigen present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from recombinant HBV core antigen and sample concentration is then determined.

Reagents And Materials Provided

1. Anti-HBVcAg Antibody Coated Plate: One strip well 96-well plate.
2. FITC-Conjugated Anti-HBVcAg Monoclonal Antibody: One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody: One 20 µL vial.
4. Assay Diluent: One 50 mL bottle.
5. Triton X-100 Solution: One 15 mL bottle containing 5% Triton X-100 in TBS.
6. 10X Wash Buffer: One 100 mL bottle.
7. Substrate Solution: One 12 mL amber bottle.

8. Stop Solution: One 12 mL bottle
9. Recombinant HBVcAg Standard: One 100 µL vial of 10 µg/mL recombinant HBV Core Antigen in PBS containing BSA.

Materials Required But Not Supplied

1. HBV Sample: purified virus or unpurified viral supernatant
2. Cell Culture Centrifuge
3. 0.45 µm filter
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store recombinant HBVcAg Standard at -20°C and avoid freeze/thaw. Store all other components at 4°C.

Specimen Collection And Preparation

1. (Optional) Dilute HBV sample in culture medium. Include culture medium as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of Triton X-100 Solution, Vortex well.
3. Incubate 30 minutes at 37°C.

Note: For samples that contain anti-HBVcAg antibody, to release HBVcAg from the virion and to inactivate anti-HBVcAg antibodies, samples should be incubated at 56°C for 30 min.

Reagent Preparation

1X Wash Buffer:

Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.

FITC-Conjugated Anti-HBVcAg Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody:

Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Solution:

1. Prepare a dilution series of Recombinant HBVcAg Standard in the concentration range of 100 ng/mL – 1 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	HBVcAg Standard (µL)	Assay Diluent (µL)	HBVcAg (ng/mL)
1	10	990	100
2	500 of Tube #1	500	50
3	500 of Tube #2	500	25
4	500 of Tube #3	500	12.5
5	500 of Tube #4	500	6.25
6	500 of Tube #5	500	3.125
7	500 of Tube #6	500	1.5625
8	0	500	0

2. Transfer 225 µL of each dilution to a microcentrifuge tube containing 25 µL of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

Assay Procedure

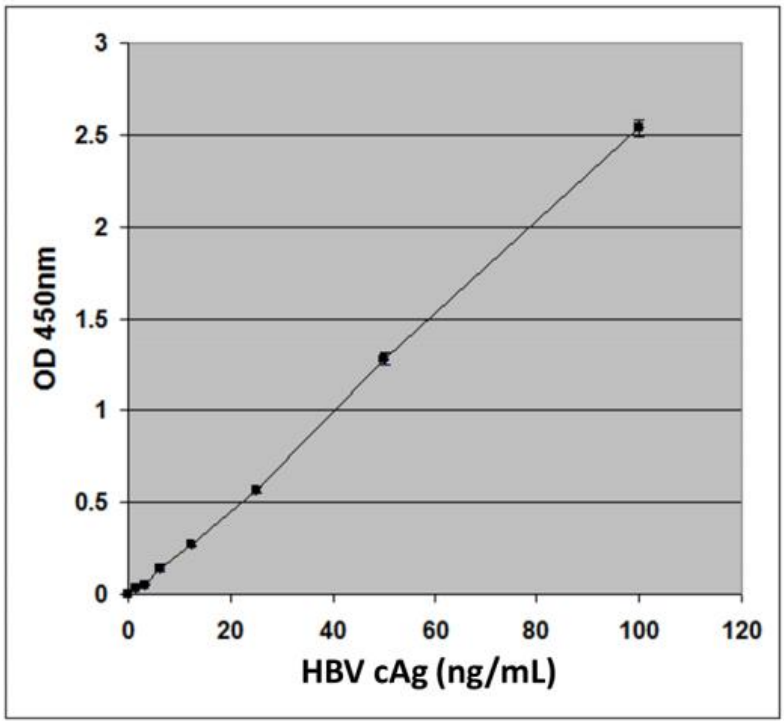
1. Prepare and mix all reagents thoroughly before use.
2. Each HBV sample, HBVcAg standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of inactivated sample or HBVcAg standard to Anti-HBVcAg Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37°C for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-HBVcAg Monoclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation. 13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Typical Standard Curve

The following figures demonstrate typical HBVcAg ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.



Sensitivity

1 ng/mL

References

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