

User's Manual and Instructions

ELISA Kit for IgM Antibody to Hepatitis B Core Antigen

Catalog No.: KO31005096

[NAME AND INTENDED USE]

ELISA Kit for IgM Antibody to Hepatitis B Core Antigen is an *in vitro* enzyme immunoassay for the detection of Anti-HBc-IgM in human serum or plasma.

[PRINCIPLE]

The purified Anti- μ -chain is coated on the solid phase of multi-wells. Serum sample, HBcAg and Horseradish peroxidase labeled with Anti-HBc (conjugated) are added to coated wells. After incubation, if Anti-HBc-IgM is present in the sample, a complex of Anti- μ -chain-Anti-HBc-IgM-HBcAg-Anti-HBc labeled with HRP will form. Wash wells to remove other unbounded serum components, incubate with substrates (TMB) to form a colored product, and measure the absorbance at 450nm to indicate the presence or absence of Anti-HBc-IgM in the sample. The test is special, sensitive, reproducible and easy to operate.

[STORAGE AND STABILITY]

Store the kit at 2~8°C. The kit is stable within 12 months after it is received.

[MATERIALS PROVIDED]

1. Anti- μ -chain Coated Microwell Plate	1 block (96wells)
2. Enzyme Conjugant	1 bottle (6ml)
3. Positive Control Serum	1 vial (0.5ml)
4. Negative Control Serum	1 vial (0.5ml)
5. Wash Buffer (1:20 dilution prior to use)	1 bottle (30ml)
6. Substrate A	1 bottle (6ml)
7. Substrate B	1 bottle (6ml)
8. Stop Solution	1 bottle (6ml)
9. HBcAg	1 bottle (6ml)
10. Seal Paper	2 pieces

[PRECAUTIONS]

1. Shake the bottled reagents well before use, discard 1~2 drops and drop vertically.
2. Bring ***ELISA Kit for IgM Antibody to Hepatitis B Core Antigen*** (all reagents), and samples to room temperature before use (approximately 30 minutes), put the remained reagents to the sealed pouch, and return to 2~8°C in time.
3. The NaN₃ can't be used to preserve the reagents.
4. Do not interchange reagents between kit lots.
5. Results should be read out within 10 minutes.
6. Resolve the concentrated wash solution at 37°C if crystals appear.
7. Handle all reagents, samples and controls as if capable of transmitting an infectious agent. It is recommended that these reagents and samples be handled using established good laboratory working practices.
8. The seal paper can't be used repeatedly.
9. Dilute the wash solution with distilled water to 1: 20 prior to use.
10. Once wells were opened, they must be used in one month.
11. Do not use kit beyond its expiration date. The date is printed on kit boxes.
12. The shelf life is 12 months.

[TEST PROCEDURE]

1. Dilute the sample with physiological saline solution to 1:1000.

2. Set one blank, two positive and two negative controls for each test, add 100 μ l serum sample, positive and negative control serum into the coated wells, seal the wells with seal paper, and incubate for 30 minutes at 37°C.
3. Discard the liquid in the coated wells and bring them to dry. Fill the wells with wash solution, discard the liquid, and bring them to dry. Repeat 3 times.
4. Add one drop (approximately 0.05ml) of enzyme conjugant and HBcAg into the same coated wells (The blank well is omitted), mix thoroughly, seal the wells with seal paper, and incubate for 30 minutes at 37°C.
5. Discard the liquid in the coated wells and bring them to dry. Fill the wells with wash solution, lay aside for 30 seconds, discard the liquid, and bring them to dry. Repeat 5 times.
6. Add one drop (approximately 0.05ml) of substrate A and B respectively to each well, mix thoroughly, and incubate for 10 minutes at 37°C.
7. Add one drop (approximately 0.05ml) of stop solution into each well, mix thoroughly, and measure the absorbance at 450nm against the blank.

[INTERPRETATION OF RESULTS]

Colorimetric Method

Cut Off Value calculation:

COV = the average OD of negative controls \times 4

Positive OD₄₅₀ of sample \geq COV

Negative OD₄₅₀ of sample $<$ COV

Invalid If the OD of positive control is below 0.80, the result is invalid. In any event, repeat the test. If the problem persists, contact the local distributor.

Notes If the absorbance of negative controls is below 0.05, calculate it as 0.05. If the absorbance of negative controls is above 0.05, calculate it as its original value.

[PERFORMANCE CHARACTERISTICS]

Sensitivity 2NCU/ml (the Biological Reference Reagents of Chinese Clinical Test Center), OD \geq 0.105

Specificity the average OD of 30 normal negative samples \leq 0.050

Precision CV(%) \leq 15% (n=10)

This Kit is for Research Use Only