

User's Manual and Instructions

ELISA Kit for Antibody to Hepatitis Be Antigen

Catalog No.: KO31007096

INTENDED USE

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

PRINCIPLE

The purified Anti-HBe is coated on the solid phase of multi-wells. Serum sample, recombinant HBeAg and Horseradish peroxidase labeled with Anti-HBe (conjugated) are added to coated wells, and form competitive combination. After incubation, if Anti-HBe content is high in the sample, a complex of HBeAg-Anti-HBe-Anti-HBe labeled with HRP will form. Wash wells to remove the complex, and incubate with substrates (TMB), subsequently there is no color change or little color change. If Anti-HBe is not present in the sample, there is much color change. The test is special, sensitive, reproducible and easy to operate.

STORAGE AND STABILITY

Store the kit at 2-8°C. The kit is stable unopened for 12 months upon receiving it.

MATERIALS PROVIDED

1. Anti-HBe Coated Microwell Plate	1 block (96 wells)
2. Enzyme Conjugant	1 bottle (6.5 ml)
3. Positive Control Serum	1 vial (1 ml)
4. Negative Control Serum	1 vial (1 ml)
5. Wash Buffer (1:20 dilution prior to use)	1 bottle (50 ml)
6. Substrate A	1 bottle (8.5ml)
7. Substrate B	1 bottle (8.5 ml)
8. Stop Solution	1 bottle (8.5ml)
9. Seal Paper	2 pieces

PRECAUTIONS

1. The samples should be fresh and avoid hemolysis, bacterial growth, and repetitive freeze-thaw cycle.
2. Do not interchange reagents between different lots.
3. The seal paper CANNOT be used repeatedly.
4. Mix reagents well before use. If crystal form in certain reagents, such as wash buffer, warm bottle/vials to redissolve. Mix well.
5. Follow the instruction provided in the manual, especially for incubation temperature and reaction time. All pipetting devices should be used with care and calibrated regularly following the manufacturer's instructions.
6. Put the remaining reagents in the sealed pouch, and return them to 2-8°C for short period of storage.
7. To prevent cross-contamination, wear gloves and lab coats throughout the procedure, and disinfection spills immediately. Dispose of all samples and materials used to perform the test. To disinfect used samples and materials before disposal, one should autoclave at 121°C or use 5.0 g/L liquid sodium hypochlorite solution (The positive control serum in the kit has been inactivated already).

ASSAY PROCEDURE

1. For each test, set one blank, two positive and two negative controls, add 0.05ml serum sample, positive and negative control serum into the coated wells, then add one drop (approximately 0.05ml) of enzyme conjugant into the same coated wells (The blank well is omitted), mix thoroughly, cover wells with seal paper, and incubate for 30-60 minutes at 37°C.
2. **Manual Wash Procedure:** Discard the liquid in the coated wells and bring them to dry. Fill the wells with 300ul wash buffer, discard the liquid. Repeat 5 times and then bring them to dry. **Automatic Wash Procedure:** Select the automatic operations of washing 5 times and bring them to dry after the operation.
3. Add one drop (approximately 0.05ml) of substrate A and B respectively to each well, seal the plate with seal paper, mix thoroughly, and incubate for 10-15 minutes at 37°C. Avoid exposure to light.
4. Add one drop (approximately 0.05ml) of stop solution into each well, mix thoroughly to terminate the reaction. Measure the absorbance at 450nm against the blank or measure the absorbance at 450nm/630-690 nm.

INTERPRETATION OF RESULTS

Colorimetric Method

Cut Off Value calculation:

COV = the average OD of negative controls × 0.5

Positive OD₄₅₀ of sample < COV

Negative OD₄₅₀ of sample ≥ COV

Invalid If the OD of positive control is below than 0.2, or the OD of negative control is greater or equal to 1.5, the result is invalid. In any event, repeat the test.

PERFORMANCE CHARACTERISTICS

Sensitivity 4NCU/ml (the Biological Reference Reagents of Chinese Clinical Test Center), OD ≤ 0.600

Specificity the average OD of 20 normal negative samples ≥ 2.000

Precision CV(%) ≤ 20%

This Kit is for Research Use Only