

## User's Manual and Instructions

### ELISA Kit for Hepatitis Be Antigen

**Catalog No.: KO31006096**

#### INTENDED USE

\*\*\*ELISA Kit for Hepatitis B e Antigen\*\*\* is an *in vitro* enzyme immunoassay for the detection of HBeAg in human serum or plasma.

#### PRINCIPLE

The purified Anti-HBe is coated on the solid phase of multi-wells. Serum sample and Horseradish peroxidase labeled with Anti-HBe (conjugated) are added to coated wells. After incubation, if HBeAg is present in the sample, a complex of Anti-HBe-HBeAg-Anti-HBe 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 HBeAg in the sample. The test is specific, sensitive, reproducible and easy to operate.

#### STORAGE AND STABILITY

Store the kit at 2~8℃. The kit is stable unopened for 12 months upon receiving it.

#### MATERIALS PROVIDED

1. Anti-HBe Coated Microwell Plate	1 block (96wells)
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 (8.5 ml)
7. Substrate B	1 bottle (8.5 ml)
8. Stop Solution	1 bottle (8.5 ml)
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℃ 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℃ 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℃.
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℃. 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 × 2.1

**Positive** OD<sub>450</sub> of sample ≥ COV

**Negative** OD<sub>450</sub> of sample < COV

**Invalid** If the average OD of negative controls ≥ 0.1, the result is invalid. In this event, repeat the test. If 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 ≥ 1.500

**Specificity** the average OD of 20 normal negative samples ≤ 0.030

**Precision** CV(%) ≤ 15%

**This Kit is for Research Use Only**