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User's Manual and Instructions

ELISA Kit for Hepatitis B Surface Antigen (ELISA)

Catalog No.: KO31003096

[NAME AND INTENDED USE]

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

[PRINCIPLE]

The purified monoclonal Anti-HBs is coated on the solid phase of multi-wells. Serum sample and Horseradish peroxidase labeled with Anti-HBs (conjugated) are added to coated wells. After incubation, if HBsAg is present in the sample, a complex of Anti-HBs - HBsAg - Anti-HBs 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 HBsAg 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 within 12 months after it is received.

[MATERIALS PROVIDED]

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

[SAMPLE COLLECTION AND PRESERVATION]

Blood serum samples are routinely prepared form vein. Blood plasma sample are routinely prepared with routine amount of anticoagulant such as heparin or sodium citrate. Sample can be stored at 4℃ if tested within five days. Sample can be stored at -20℃ at least for 3 months. Avoid hemolysis and repetitive freeze and thaw of samples. Samples with cloud or precipitation should be centrifuged or filtered before test. Prevent serum from bacteria contamination during collection and storage.

[TEST PROCEDURE]

1. Bring *** ĖLISA Kit for Hepatitis B Surface Antigen *** (all reagents), and samples to room temperature before use (approximately 30 minutes).
2. Dilute concentrated wash buffer 1:20 with ddH2O
3. For each test, set one blank without sample and enzyme conjugate, two positive and two negative controls. Add 50 µl positive and negative control serum into positive and negative control wells respectively . Add 50 µl samples in each other well
4. Add 50 ul of enzyme conjugate into each test well. Cover wells with seal paper, mix well, and incubate for 60 minutes at 37℃.
5. Discard the liquid in all wells and fill the wells with wash solution (300µl per well). Lay aside for 10 seconds, discard the liquid in all wells and fill the wells with wash solution. Repeat 5 times and dry wells after last wash.
6. Add 50 µl Substrate A and 50 µl Substrate B into each well, Cover with seal paper, mix well, and incubate at 37℃ for 15 minutes away from light.
7. Add 50 µl Stop Solution into each well, mix well.
8. Measure the absorbance at 450nm against the blank, or measure the absorbance at 450nm/630nm.

[INTERPRETATION OF RESULTS]

Colorimetric Method

Cut Off Value calculation:

COV = the average OD of negative controls × 2.1

Positive OD_{450} of sample ≥ COV

Negative OD_{450} of sample < COV

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.

[PRECAUTIONS]

1. The samples should be fresh, avoid hemolysis, bacteria growing, and repetitive freeze and thaw.
2. Do not interchange reagents between kit lots. The seal paper can't be used repeatedly.
3. Mix reagents well before use. If crystal form in certain reagents, such as wash buffer, it can be used without problems after warm up and mix well.
4. Follow instruction exactly during assay, especially in temperature and time for reactions. All pipetting devices should be used with care and calibrated regularly following the manufacturer's instructions.
5. Put the remained reagents to the sealed pouch, and return to 2~8℃ in time.
6. To prevent cross-contamination, wear gloves and working suits throughout the procedure, and execute the disinfection and isolation regulations strictly. Dispose of all samples and materials used to perform the test. The 5.0g/L liquid sodium hypochlorite solution or 121℃ high pressure steam may be used to disinfect samples and materials before disposal

[PERFORMANCE CHARACTERISTICS]

Sensitivity 0.5ng/ml(adr), OD≥0.105

Specificity the average OD of 20 normal negative samples and 3 Albumin Fraktion (2%)≤0.030

Precision CV(%)≤15% (n=10)

This Kit is for Research Use Only