Technical Manual

Human Hepatitis B Virus (HBV) Large Protein ELISA Kit

- Catalogue Code: HDES0061
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Sandwich-ELISA as the method to detect the Hepatitis B Virus large protein (HBV-LP) antigen in human serum or plasma. The ELISA Microtiter plate provided in this kit has been pre-coated with anti-HBV-LP antibody. Add samples to the ELISA Microtiter plate wells, The HBV-LP antigen in samples will be combined with the anti- HBV-LP antibody. Free components are washed away. Then add HRP conjugated anti-HBs, the combined HBV-LP antigen will then specifically combine with the HRP conjugated anti-HBs. The substrate reagent is added to initiate the color developing reaction. The presence of HBV- HBV-LP antigen can be determined according to the absorbance value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specifications
ELISA Micro-plate	96 wells
HRP Conjugate	10 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

3. Experimental instrument

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper

4. Sample preparation

1. **Serum/plasma**: Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive. Samples can be stored at 2~8° C for one week and stored at -20 ° C for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
2. Anticoagulant (EDTA, sodium citrate and heparin sodium) in samples do not affect the result of the experiment in general. Endogenous interference substances in serum such as blood fat, cholerythrin, and hemoglobin will not affect the results.
3. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

5. Assay procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Set 2 wells for negative control and 2 wells for positive control. **Samples need test in duplicate**.
2. **Add sample:**
   (1) Add 50 μL of **Positive/Negative Control** respectively to **Positive/Negative Control** wells, keep the blank control well empty.
   (2) Add 50 μL of **Serum/plasma** into sample well, mix fully.
3. **Incubate:** cover the plate with plate sealer, mix fully. Incubate at 37° C for 30 min in shading light.
4. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60s each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 μL of **HRP Conjugate** to each well except the blank control well. Cover the plate with plate sealer. Incubate at 37° C for 30min in shading light.
6. **Wash:** repeat step 4.
7. **Color Development:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate at 37° C for 10 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. **OD Measurement:** set the microplate reader wavelength at 450 nm (it is recommended to

set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not needed when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 10 min.**

6. Reference value

Normally, positive control (PC): $A_{450} \geq 1.0$ and average A value of negative control (NC): $A_{450} < 0.1$.

7. Interpretation of the results

Cut Off(C.0) = 2.1 × average A value of negative control (NC) (when average A450 of NC $<$ 0.05, calculate at 0.05; while average A value of NC $\geq$ 0.05, calculate at the actual value).

1. Positive result: average A value of sample $\geq$ Cut Off.
2. Negative result: average A value of sample $<$ Cut Off.

8. Limitations of test method

1. This test is only used as the qualitative detection of HBV-LP in serum and plasma of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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