Technical Manual

Human Hepatitis B Virus (HBV) E Antigen ELISA Kit

- Catalogue Code: HDES0084
- Antibody ELISA Kit
- Research Use Only

1. Test principle

Using multiple monoclonal HBeAb to coat the reaction plate, add the test specimen along with HBeAb-HRP. When HBeAg is present in the specimen, it binds to the coated HBeAb and forms a complex with HBeAb-HRP, creating an HBeAb-HBeAg-HBeAb-HRP complex. Adding TMB substrate produces a colorimetric reaction; conversely, no colorimetric reaction occurs if HBeAg is absent. The presence of HBeAg can be determined by measuring the absorbance value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
20X Concentrated Wash Buffer	30 mL
HRP Conjugate	6 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

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other supplies required

- Microplate reader with 450nm wavelength filter
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37°C Incubator
- Deionized water
- Absorbent paper

4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.

3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0084. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0084 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and expiry date

Store protected from light at 2 to 8°C. Do not freeze.

Please store the opened plate at 2~8° C, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the box.

5. Requirements of sample

1. **Serum/plasma:** Human serum and plasma can be used as detected sample. Fresh collected samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive.
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 lipids, cholerythrin, hemoglobin, rheumatoid factors positive samples may not affect the results.
3. 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.
4. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

6. 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 1 well for blank control, 2 wells for negative control

and 3 wells for positive control. (Blank well is not necessary for dual-wavelength detection)

2. **Add sample:**
   a) Add 50 μL of **negative control**/**positive control** to **negative control**/**positive control** wells, keep the blank control well empty.
   b) Add 50 μL of serum sample to other sample wells.
   c) Gently tap the plate to mix thoroughly.
3. **HRP conjugate:** add 50 μL of **HRP Conjugate** to each well except the blank control well.
4. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes at 37°C in shading light.
5. **Wash:** after incubation, 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).
6. **Add Substrate:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 15 minutes at 37°C in shading light.
7. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
8. **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 essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 30 min.**

7. Interpretation of test results

Use each test result independently. Determine the result according to the Cut Off value.
Cut Off(C.0) = 2.1 × average A value of negative control(NC) (when average A_{450} of NC ＜0.05, calculate at 0.05; while average A_{450} of NC ≥ 0.05, calculate at the actual value).
Under normal circumstances, the A_{450} of NC≤ 0.1 (if the OD value of one negative control well is greater than 0.1, the result of that well should be discarded; if the OD values of two negative control wells are greater than 0.1, the experiment should be repeated); The A_{450} of PC≥ 0.8.

1. Positive result: average A value of sample ≥ Cut Off.
2. Negative result: average A value of sample ＜ Cut Off.

8. Limitations

1. This kit is only for qualitative detection of Hepatitis B Virus E (HBe) Antigen in 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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