Technical Manual

Human Hepatitis A Virus (HAV) IgG ELISA Kit

- Catalogue Code: HDES0045
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the Hepatitis A Virus (HAV) IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified HAV antigen. Samples are added to the ELISA Microtiter plate wells and the HAV-IgG in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugated Mouse-anti-human IgG antibody is added to each well and react with the compound to form “antigen- antibody-HRP antibody” compound. The TMB substrate is added to initiate the color developing reaction. The presence of HAV-IgG can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugate	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

3. Other materials required but not supplied

- Micro-plate 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
- Loading slot for Wash Buffer

4. Notes

1. 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.

2. 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.
3. The ELISA 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.
4. 20×Concentrated Wash Buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature to dissolve completely before use.
5. Each well must be filled with liquid when washing to prevent residual free enzyme.
6. The tested sample should be kept fresh.
7. The results shall depend on the readings of the Micro-plate Reader.
8. Do not use components from different batches of kit.

5. Storage and expiry date

Store unopened at 2 to 8° C. Do not freeze.
Please store the opened kit at 2-8° C, protect from light and moisture. The shelf life of the opened kit is up to 1 months.
Expiry date: expiration date is on the box.

6. Sample preparation

1. **Serum:** Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated.
2. Avoid of samples with hyperlipidemia (triglyceride > 20 g/L), hemolysis (hemoglobin > 10 g/L) or jaundice (bilirubin > 0.2 g/L). Obviously contaminated samples can't be detected. Do not use heated inactivated samples. Heat inactivation will degrade antibodies.
3. There should be no microbes in the sample. Samples can be stored at 2-8° C for one week. If samples not tested in a week, store them at -20° C and avoid freeze-thaw cycles.
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.

7. 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 control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control, 3 wells for negative control and 2 wells for positive control. **Samples need test in duplicate** (Blank well is not necessary

for dual-wavelength detection).

2. **Add sample:**
   a) Add 100 μL of **Negative/Positive Control** respectively to 3 negative control wells, 2 positive control wells, keep the blank control well empty.
   b) Dilute the tested Serum with **Sample Diluent** at 1:10 into sample well (add 100 μL of Sample Diluent and add 10 μL of Serum sample), mix fully.
3. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37° C 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 30-60 sec 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.
6. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes at 37° C in shading light.
7. **Wash:** repeat step 4.
8. **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 min at 37° C in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
10. **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.

8. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: $A_{450} \leq 0.08$; positive control (PC): $A_{450} > 0.80$ and average A value of negative control (NC): $A_{450} \leq 0.08$.

9. Interpretation of the results

Cut Off = 0.10 + 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).

1. Positive result: A_{450} of Sample ≥ Cut Off.
2. Negative result: A_{450} of Sample < Cut Off.
3. Negative result indicates no HAV-IgG antibody detected in samples, while positive result means the opposite.

10. Limitations of test method

1. This test is only used as the qualitative detection of HAV-IgG in serum 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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