HAV-Ig (hepatitis A virus Antibody) ELISA Kit

Catalogue No.: EU3600

Size: 96T

Reactivity: Universal

Application: This immunoassay kit allows for the qualitative determination of HAV-Ig in serum or plasma.

Storage: 2-8°C.

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	8 ×12	2-8°C /-20°C
HAV-Ig Positive Control	200ul×1	2-8°C
HAV-Ig Negative Control	200ul×1	2-8°C
Sample dilution buffer	20ml×1	2-8°C
HRP- HAV IgG	6ml×1	2-8°C (Avoid Direct Light)
TMB substrate	10ml×1	2-8°C (Avoid Direct Light)
Stop solution	5ml×1	2-8°C
Wash buffer (25X)	30ml×1	2-8°C
Plate Sealer	3pieces	
Product Description	1 copy	

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Principle of the Assay

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with HAV-Ag. During the reaction, HAV-Ig present in the sample to compete with HRP- HAV-IgG for a fixed amount HAV antigens pre-coated in the wells. Excess conjugate and unbound sample or standard are washed from the plate. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of the target substance was inversely proportional to the OD450 value.

Precautions for Use

1.After opening and before using, keep plate dry.

2. Before using the Kit, balance the reagents at room temperature at least 30 mins.

3. Storage TMB reagents avoid light.

4. Washing process is very important, not fully wash easily cause a false positive.

5. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.

6. Don't reuse tips and tubes to avoid cross contamination.

7. Avoid using the reagents from different batches together.

Material Required But Not Supplied

1.Microplate reader (wavelength: 450nm)

2.37°C incubator

3.Automated plate washer

4.Precision single and multi-channel pipette and disposable tips

5.Clean tubes and Eppendorf tubes

6.Deionized or distilled water

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Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automated Washing

Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.
- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Wash Buffer Preparation:

Dilute 30mL of Concentrated Wash Buffer to 750 mL of Wash Buffer with deionized or distilled water.

Assay Procedure

1. Label the sample wells, 3 Negative Controls, 2 Positive Controls and 1 blank well.

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2. First add 100ul Sample dilution buffer to each wells (except blank well); Then add 10ul sample, 10ul positive control and 10ul negative control to respective wells, immediately add 50ul HRP-HAV-IgG, gently mix for 1 minute, Seal the plate with a cover and incubate at 37°C for 1 hour.

3. Remove the cover, wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1 minute each time.

4. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark for 15 min. And the shades of blue can be seen in the Negative Controls. Positive Controls wells show no obvious color.

5. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

6. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

Data Analysis

NCx: Mean Absorbance of Negative Controls

Cutoff Value = **NCx x 0.5**

Quality control

Blank well： A <0.1

Negative control: A > 0.8

Positive control: A < 0.2

Interpretations of the results

Sample with absorbance values ≥ Cutoff Value are NON-REACTIVE and are considered NEGATIVE for HAV-Ig.

Sample with absorbance values < Cutoff Value are considered POSITIVE for HAV-Ig.

Borderline (S/CO =0.9-1.1): Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline. Retesting of these samples in duplicates is recommended. Repeatedly reactive samples could be considered positive for antibodies to HAV.

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