Technical Manual

Human HAV-IgG (hepatitis A virus-Immunoglobulin G) ELISA Kit

- Catalogue Code: HUFI04728
- Indirect ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

{Alias}

Uniprot:

{}

Detection method:

Indirect

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

{Species}

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Indirect ELISA kit is a highly sensitive assay for the qualitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

This kit was based on indirect enzyme-linked immune-sorbent assay technology. CA16-Ag was pre-coated onto 96-well plates. The test samples are added to the wells, unbound conjugates are washed away with the wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalysed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays including:

No.	Component	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 12	2-8°C/-20°C
2	CA16-IgG Positive Control	1ml x 1	2-8°C
3	CA16-IgG Negative Control	1ml x 1	2-8°C
4	Sample dilution buffer	12ml x 1	2-8°C
5	HRP- Conjugates	12ml x1	2-8°C
6	TMB substrate A	6ml x 1	2-8°C (Avoid Direct Light)
7	TMB substrate B	6ml x1	2-8°C(Avoid Direct Light)
8	Stop solution	6ml x1	2-8°C
9	Wash buffer (20X)	50ml x 1	2-8°C
10	Plate Sealer	3	
11	Manual	1	

Additional materials required:

1. 37°C incubator
2. Plate Reader with 450nm filter
3. Precision pipettes and disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

Precautions

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

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

3. Before using the kit, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

5. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolyzed samples are not suitable for use with this kit.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolyzed samples are not suitable for this assay.

6. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: Matrix components in the sample will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

1. Wash Buffer:

Dilute 50mL of Concentrated Wash Buffer into 950 mL of Wash Buffer with deionized or distilled water

7. Assay Procedure

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

2. Add 100μL Negative Controls and Positive Controls to each well (except blank well).

3. Add 100μL sample dilution buffer to sample wells and then add 10μL sample serum or plasma. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 30 min.

4. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1- 2 minute each time.

5. Add 100μL HRP-Conjugates to each well, except blank well

6. Seal the plate with a cover and incubate at 37°C for 30 min.

7. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1- 2 minute each time.

8. Add 50μl of TMB substrate A and 50μl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark for 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

9. Add 50μl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately. 10. 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).

8. Data Analysis

Cut-off Value = **NCx**+0.1

NCx: Mean Absorbance of Negative Control (When the negative mean A value is less than 0.05, it is calculated as 0.05.When the negative mean A value is greater than or equal to 0.05, it is calculated according to the actual value.)

2. Determination of results

Sample with absorbance values ≤ Cutoff Value are NON-REACTIVE and are considered NEGATIVE for CA16- IgG. Sample with absorbance values > Cutoff Value are considered POSITIVE for CA16-IgG.

3. Quality control

The blank well (only adding TMB and Stop solution) should not be greater than 0.08. The positive control (PC) A value was greater than 0.80. The negative mean A value was less than 0.1.

Notes

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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