Technical Manual

Human Anti-ITIH4 (Inter-alpha-trypsin inhibitor heavy chain H4antibody ELISA Kit

- Catalogue Code: AEFI01103
- Indirect ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Detection method:

Indirect

Sample Type:

Serum, plasma, cell culture supernatant, cell or tissue lysates and other biological samples

Reactivity:

Human

Range:

0.781-50 ng/ml

Sensitivity:

0.469ng/ml

Storage:

2-8°C for 6 months (for sealed box)

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. The sealed kit can be stored at 2-8 ℃. The storage condition for opened kit is specified in the “Kit Contents” section. 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 quantitative measurement of a specific analyte in the following samples: serum, plasma, cell culture supernatant and other biological samples.

How do our ELISA kits work?

This kit was based on indirect enzyme-linked immune-sorbent assay technology. Antigen was pre-coated onto 96-well plates. And the biotin conjugated antibody was used as detection antibody. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with 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. The O.D. absorbance can be read 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.

The sealed kit can be stored at 2-8 ℃. The storage condition for opened kit is specified in the table below

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 6	8 x 12	2-8°C for 1 month/-20°C or 6 months
2	Lyophilized Standard	1 vial	2 vials	2-8°C for 1 month/-20°C or 6 months
3	Sample Dilution Buffer	10 mL	20 mL	2-8°C
4	Biotin-labelled Antibody (Concentrated 100X)	60 uL	120 uL	2-8°C (Avoid Direct Light)
5	Antibody Dilution Buffer	5 mL	10 mL	2-8°C
6	HRP-Streptavidin Conjugate (SABC)	60 uL	120 uL	2-8°C (Avoid Direct Light)
7	SABC Dilution Buffer	5 mL	10 mL	2-8°C
8	TMB Substrate	5 mL	10 mL	2-8°C (Avoid direct light)
9	Stop Solution	5ml	10ml	2-8°C
10	Wash Buffer (25x)	15 mL	30 mL	2-8°C
11	Plate Sealer	3 pieces	5 pieces	
12	Manual	1	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

5. Sample Preparation

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: EDTA-Na2/K2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 4°C within 30 minutes after collection. Collect the supernatant (plasma fraction and assay promptly or aliquot and store the samples at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

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

Cell culture supernatant: Collect the supernatant: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect clarified cell culture supernatant to detect immediately or aliquot the supernatant and store it at -80°C for future's assay.

Cell lysates:

Suspension Cell Lysate: Centrifuge at 2500 rpm at 4℃ for 5 minutes and collect cells. Wash with pre- cooled PBS. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

Adherent Cell Lysate: Absorb supernatant and add pre-cooled PBS to wash three times. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 4°C for 10 minutes. Add the supernatant into EP tube to assess immediately or aliquot the supernatant and store it at - 80°C for future's assay.

Tissue Homogenates: Generally, tissue samples are required to be made into homogenization.

1. Place the target tissue on ice. Remove residual blood by washing tissue with pre-cooled PBS buffer (0.01M, pH=7.4). Then weigh for usage.

2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. It is recommended to add some protease inhibitors into the PBS (e.g. 1mM PMSF).

3. The samples can be further processed using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

4. Centrifuge the homogenates for 5 minutes at 5000×g. Collect the supernatant to detect immediately or aliquot the supernatant and store it at -20°C or -80°C for future's assay.

5. Determine total protein concentration by BCA assay kit (BN01031) for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, PH7.3.

Other Biological Samples: Centrifuge samples for 15 minutes at 1000 x g (4°C). Collect the supernatant to detect immediately. Or aliquot the supernatant and store it at -80°C.

Notes

- Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.
- The best sample storage condition: less than 5 days at 2-8℃; within 6 months at -20℃; within 2 years at -80℃. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15- 25℃ can decrease the effect of ice crystal (0℃) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.

- The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.
- Pretest is recommended for special samples without reference data to validate the validity.
- Recombinant protein may not match with the capture or detection antibody in the kit, resulting in the undetectable 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:

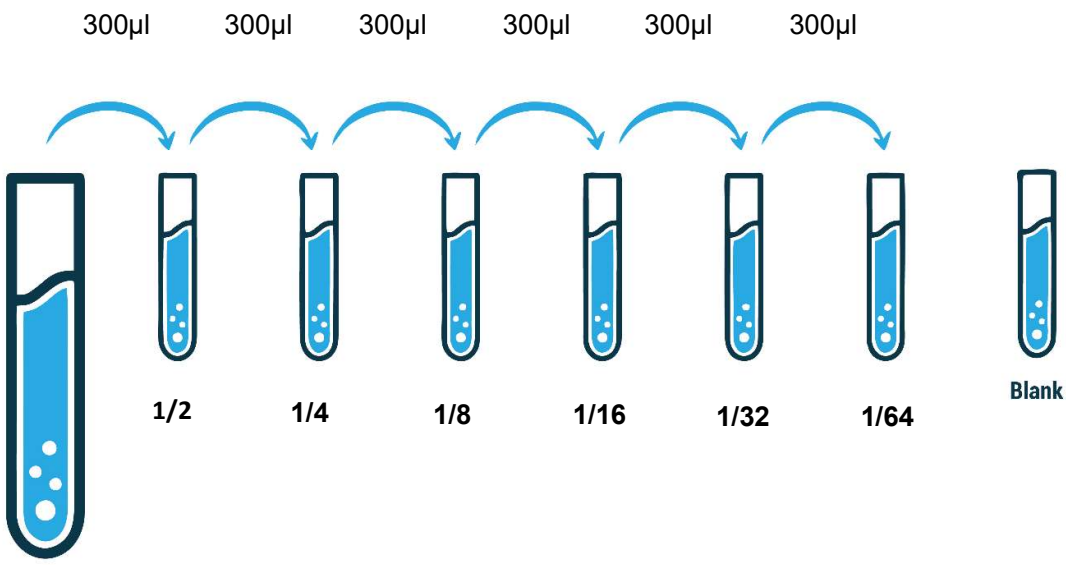
Dilute 30ml (15 ml for 48 well assay) of Concentrated Wash Buffer into 750 ml (375 ml for 48 well assay) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. If crystals have formed in the concentrate, warm at 40°C in water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.

2. Standard Dilution:

1). Centrifuge standard tube for 1min at 10000xg. Add 1ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 2 min Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)

2). Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Aliquot 300µl of the Sample dilution buffer into each tube. Add 300µl of the above (Stock Solution) standard solution into 1st tube and mix thoroughly. Transfer 300µl from 1st tube to 2nd tube and mix thoroughly. Transfer 300µl from 2nd tube to 3rd tube and mix thoroughly, and so on.

DILUTION SERIES



Stock Solution

Note: The standard solutions are best used within 2 hours. The standard solution series should be kept at 4°C for up to 12 hours.

3. Preparation of Biotin-labelled antibody working solution:

Prepare within 30 min before the experiment.

1. Calculate the total volume of the working solution: 100µl / well × quantity of wells (Allow 100-200µl more than the total volume).
2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated biotin-labeled antibody to the bottom of tube.
3. Dilute the Biotin-detection antibody with antibody dilution buffer at 1:100 and mix thoroughly (i.e. Add 1µl of biotin-labelled antibody into 99µl of antibody dilution buffer).

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

Prepare within 30 min before the experiment.

1. Calculate the total volume of the working solution: 100µl / well × quantity of wells (Allow 100-200µl more than the total volume).
2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated biotin-labeled antibody to the bottom of tube.
3. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly (i.e. Add 1µl of SABC into 99µl of SABC dilution buffer.)

7. Assay Procedure

Before adding to the wells, equilibrate the TMB substrate for at least 30 mins at 37'C. When diluting samples and reagents, ensure they are mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standard, **test sample (diluted at least ½ with Sample Dilution Buffer)** and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (blank) wells.**
2. **Standards:** Aliquot 100µl of standard solutions into the standard wells.
3. **Dilution Buffer:** Add 100µl of Sample dilution buffer into the control (blank) well.
4. **Samples:** Add 100µl of properly diluted sample into test sample wells.
5. **Incubate:** Seal the plate with a cover and incubate at 37 °C for 90 mins.
6. **Wash:** Remove the cover, aspirate the liquid from the plate and wash plate 2 times with 350 µl of Wash Buffer. **Do NOT let the wells dry completely at any time.**

7. **Biotin-labeled Antibody:** Add 100µl of Biotin-labelled antibody working solution to the bottom of each well (standard, test sample & zero wells) without touching the side walls.

8. **Incubate:** Seal the plate with a cover and incubate at 37°C for 60 mins.

9. **Wash:** Remove the cover, and wash plate 3 times with 350 µl of Wash buffer. **Let the Wash Buffer stay in the wells for 1-2 minutes for each wash.**

10. **HRP-Streptavidin Conjugate (SABC):** Add 100µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 mins.

11. **Wash:** Remove the cover and wash plate 5 times with 350 µl of Wash buffer. **Let the Wash Buffer stay in the wells for 1-2 minutes for each wash.**

12. **TMB Substrate:** Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark for 10-20 mins. (Note: This incubation time is for reference only, the optimal time should be determined by the end-user.) As soon as a blue colour develops in the first 3-4 wells (with most concentrated standards) and the other wells show no obvious colour, terminate the reaction by moving to Step 13.

13. **Stop Solution:** Add 50µl of Stop solution into each well and mix thoroughly. The colour changes into yellow immediately.

14. **OD Measurement:** Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

8. Data Analysis

Calculate using the following equation:

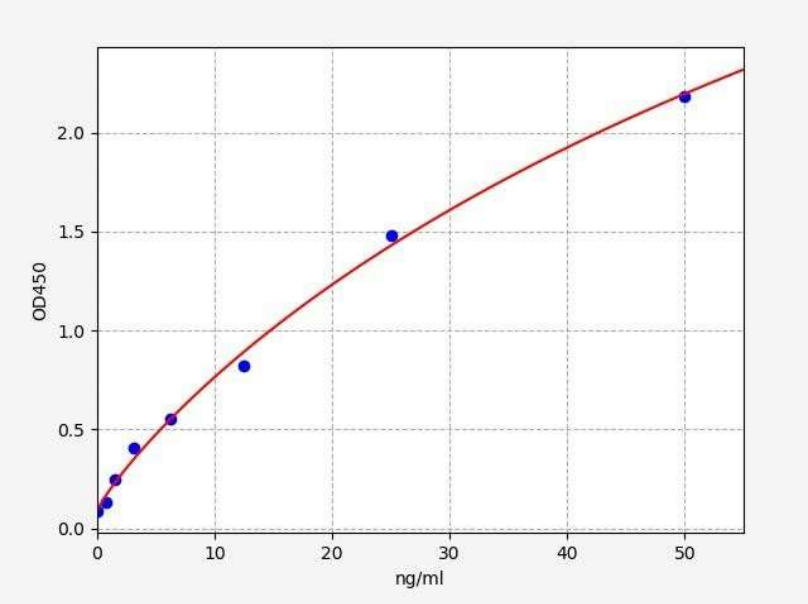
The relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well)

The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be determined from the standard curve. It is recommended to use professional software such as Curve Expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

9. Typical Data & Standard Curve

Results of a typical standard run of an ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.



Specificity

This assay has high sensitivity and excellent specificity for detection of Human anti-ITIH4 (Inter-alpha-trypsin inhibitor heavy chain H4) antibodies. No significant cross-reactivity or interference between Human anti-ITIH4 antibodies and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between anti-ITIH4 (Inter-alpha-trypsin inhibitor heavy chain H4) antibodies and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with a certain level of Anti-ITIH4 antibodies and the recovery rates were calculated by comparing the measured value to the expected amount of Anti-ITIH4 antibodies in the samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	90-103	92
EDTA Plasma(n=5)	86-105	96
Heparin Plasma(n=5)	85-101	91

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Anti-ITIH4 antibodies and their serial dilutions.

Sample	1:2	1:4	1:8
Serum(n=5)	89-100%	81-86%	81-93%
EDTA Plasma(n=5)	84-98%	90-105%	89-104%
Heparin Plasma(n=5)	85-103%	83-92%	91-100%

Stability

The stability of the Anti-ITIH4 antibody ELISA Kit is determined by the loss rate of activity. The stability test for the sealed kit was performed at 37°C and at 2-8°C. The loss rate of this kit is less than 5% within the expiration date under appropriate storage conditions.

Elisa kit(n=5)	37°C for 1 month	2-8°C for 6 months
Average (%)	80	95-100

Precision

Intra-assay Precision: samples with low, medium and high concentration were tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration were tested 20 times on three different plates.

Item	**Intra-assay Precision**			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	1.45	5.92	24.22	1.56	6.13	24.78
Standard deviation	0.08	0.31	1.33	0.07	0.33	1.44
CV(%)	5.25	5.23	5.5	4.34	5.46	5.82

Note: To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end**.**

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

Email: info@ASSAYGenie.com

Web: www.ASSAYGenie.com