Technical Manual

Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep ELISA Kit

- Catalogue Code: AEES02635
- Sandwich ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Detection method:

Sandwich

Sample Type:

Serum, Plasma, and other biological fluids

Reactivity:

Human

Range:

0.25-16 ng/mL

Sensitivity:

0.15 ng/mL

Storage:

See Kit Contents on page 5

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit according to the directions in the kit table of contents on page 5.

3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative 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?

The Assay Genie (enzyme-linked immunosorbent assays) assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, Assay Genie have developed a range of sensitive, fast, and reliable ELISA kit assays to meet and exceed those demands.

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human ICAM-1/CD54 (intercellular adhesion

molecule 1) Quickstep. Samples (or Standards) and Horseradish Peroxidase (HRP) linked antibody specific for Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstepare added to the micro- ELISA plate wells. Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep in samples (or standards) combines with the coated antibody and HRP linked detection antibody special to Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep. Excess conjugate and unbound sample or standard are washed from the plate. The substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450±2 nm. The OD value is proportional to the concentration of Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep. The concentration of Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstepin the samples is then determined by comparing the OD of the samples to the standard curve.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays, please store the reagents per conditions below.

Please note that the liquid bottles contain slightly more reagent than indicated on the label. Please use a pipette to accurately measure out required amounts.

No.	Component	48 well	96 well	Storage
1	ELISA Microplate (dismountable)	8 x 6	8 x 12	2-8°C .
2	Reference Standard	1 vial	2 vials	2-8℃, use the reconstituted standard within 24h.
3	Concentrated HRP Conjugate (x100)	30 uL	60 uL	
4	HRP-Conjugate Diluent	14 mL	14 mL	
5	Standard & Sample Dilution Buffer	20 mL	20 mL	2-8°C (Avoid Direct Light)
6	Substrate Reagent	10 mL	10 mL	2-8°C
7	Stop Solution	10 mL	10 mL	
8	Wash Buffer (25X)	30 mL	30 mL	
9	Plate Sealer	3 pieces	5 pieces	2-8°C
10	Product Description	1	1	2-8°C (Avoid Direct Light)

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. Ensure unopened and unused plates are kept dry to avoid contamination.

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

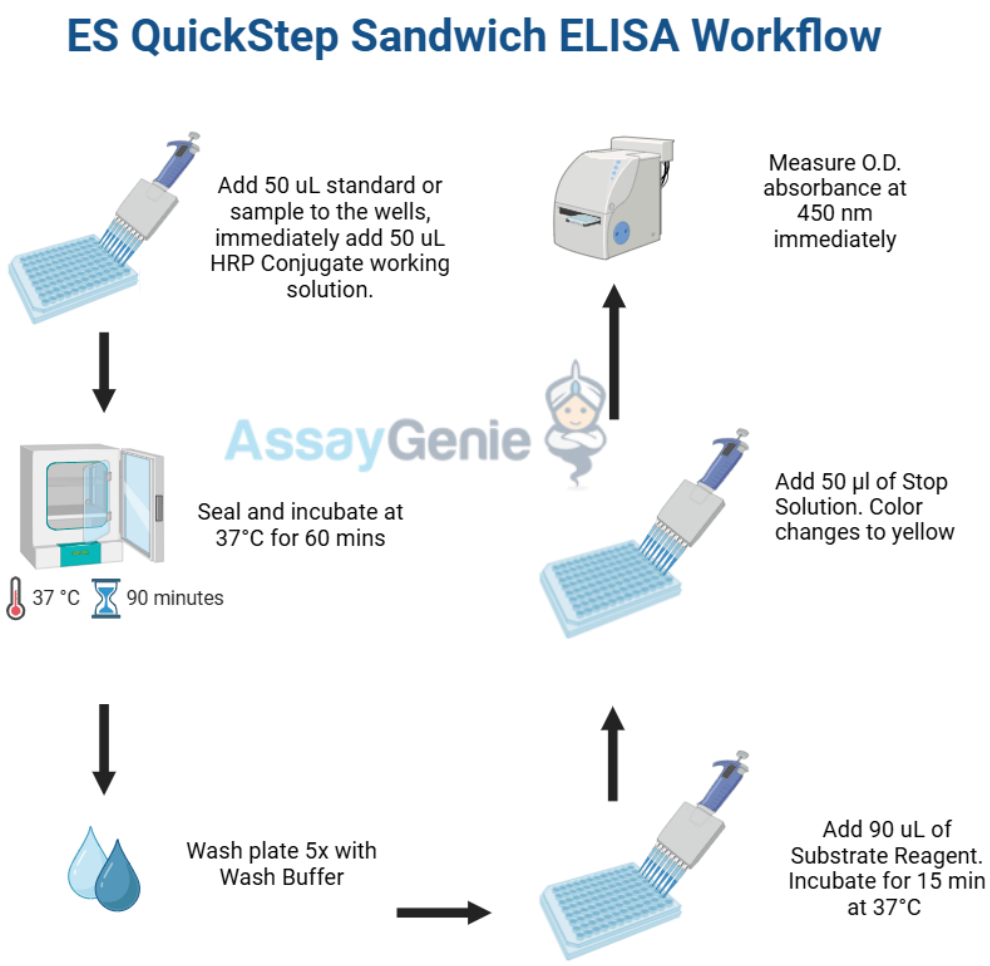
7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

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

9. Avoid using the reagents from different batches together.

10. Please wear the lab coat, mask, and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples. Please follow regulations on safety protection of biological laboratory.

5. Workflow Overview



6. Sample Preparation

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA-Na2 as anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

Recommended reagents for sample preparation: 10×EDTA Anticoagulant.

Note for sample:

1) Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.

2) Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤ 1 month) or -80℃ (≤ 3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.

3) Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

4) If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity. If a lysis buffer is used to prepare tissue homogenates or cell lysates, there is a possibility of causing a deviation due to the introduced chemical substance.

5) Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

7. 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: It is recommended to dilute normal serum/plasma samples at 5-200 fold. Due to individual differences, please estimate the concentration range of the sample in advance, and conduct a preliminary test to determine the appropriate dilution ratio of the sample

Reagent Preparation

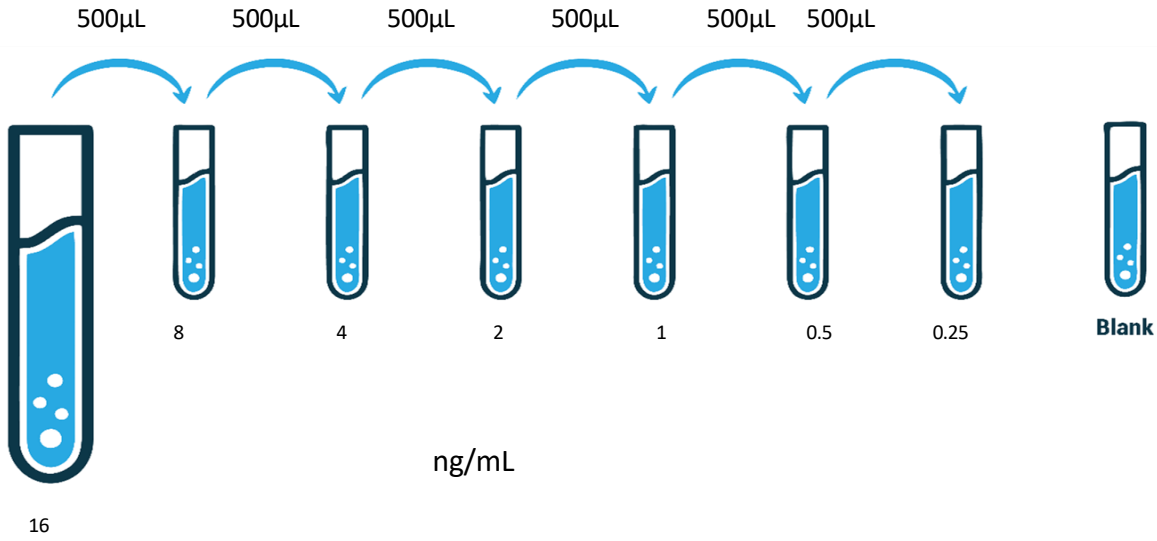
Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

1. Wash Buffer:

Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.

2. Standard Dilution:

1. **Centrifuge the standard** at 10,000×g for 1 min. Add 1 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 16 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 8 ng/mL. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.
2. **Dilution method:** Take 7 EP tubes, add 500µL of Reference Standard & Sample Diluent to each tube. Pipette 500µL of the 16 ng/mL working solution to the first tube and mix up to produce a 8 ng/mL working solution. Pipette 500µL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference.



3. **HRP Conjugate working solution:** HRP Conjugate is HRP conjugated antibody. Calculate the required amount before the experiment (50 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100×Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99). The working solution should be prepared just before use.

8. Assay Procedure

1. **Determine wells for diluted standard, blank and sample**. Add 50 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Immediately add 50 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 60 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible

2. Decant the solution from each well, add 350 µL of **wash buffer** to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

3. Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.

4. Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution

5. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

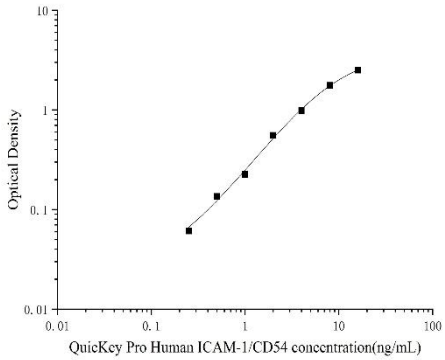
9. Data Analysis

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

10. Typical Data & Standard Curve

Standard Curve

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.



 Email: Info@assaygenie.com Web: www.AssayGenie.com

Concentration (ng/mL)	OD	Corrected OD
16	2.59	2.508
8	1.84	1.758
4	1.064	0.982
2	0.637	0.555
1	0.307	0.225
0.5	0.217	0.135
0.25	0.143	0.061
0	0.082	-

Specificity

This assay has high sensitivity and excellent specificity for detection of Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep. No significant cross-reactivity or interference between Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with a certain level of Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep and the recovery rates were calculated by comparing the measured value to the expected amount of Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep in the samples.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	88-104	96
EDTA plasma (n=5)	87-99	94
Cell culture media (n=5)	91-104	96

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Human ICAM-1/CD54 (intercellular adhesion molecule 1) Quickstep and their serial dilutions.

		Serum (n=5)	EDTA plasma (n=5)	Cell culture media (n=5)
1:2	Range (%)	89-100	95-109	87-101
	Average (%)	95	102	94
1:4	Range (%)	85-98	97-112	87-103
	Average (%)	91	103	95
1:8	Range (%)	86-98	91-106	89-102
	Average (%)	91	99	96
1:16	Range (%)	88-101	99-110	86-99
	Average (%)	93	104	92

Precision

- **Intra-Assay:** CV<8%
- **Inter-Assay:** CV<10%

11. ELISA Troubleshooting

If the ELISA result is unsatisfactory, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect order for adding reagents	Confirm the required reagent added in each step. Also repeat the assay and verify.
	Components used from different kits	Use the component included in the same kit. Also repeat the assay and verify.
	Forget to add some reagents	Verify whether the required reagent is added.
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.

Problem	Possible Causes	Solutions
Poor standard curve	Inappropriate curve fitting model	Try to plot the curve by different fitting models.
Samples without signal	The amount of sample is lower than the detection range.	Decrease dilution ratio or concentrate the sample.
	The detection target is incompatible with the buffer.	Verify the compatibility of sample storage buffer with the sample.
	Incorrect preparation of sample	Please refer to sample preparation guideline.
	Longer storage of sample or freeze- thaw cycle	Aliquot and store samples according to the assay requirement.
High CV%	Precipitate is formed in the well during staining.	Increase the dilution ratio of the sample.
	Unclean plate	Don't touch the bottom of the plate during the assay.
	Foam is found in the well.	Avoid foaming during reading in a microplate reader.
	Each well is washed unevenly.	Check whether the tube of the washer is smooth.
	Reagents are not completely mixed.	Mix all reagents completely.
	Inconsistent pipetting	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Standards are improperly reconstituted.	Before opening, shortly centrifuge the lyophilized standard tube till complete dissolution.
	Standards have been degraded.	Follow suggested storage conditions for

Notes:

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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