

Mouse ICAM-1/CD54 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE10063

Size: 96T

Sensitivity: 3.2 pg/mL

Range: 15.6-1000 pg/mL

Usage: For the quantitative detection of mouse ICAM-1/CD54 concentrations in serum, plasma, cell culture supernatant and tissue homogenate.

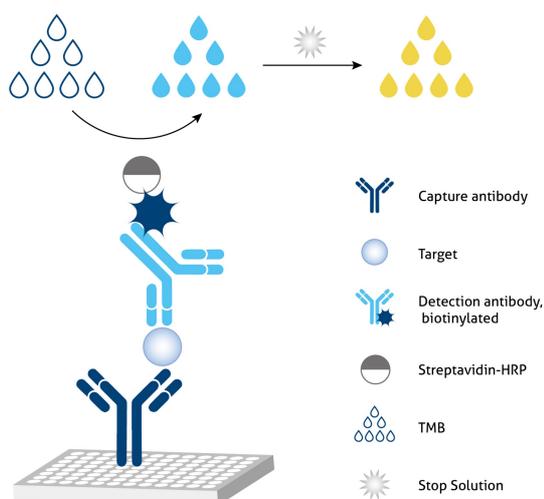
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1. Background

Intercellular adhesion molecule 1 (ICAM-1, also known as CD54) is a transmembrane glycoprotein of the immunoglobulin superfamily and is critical for the firm attachment and transmigration of leukocytes out of blood vessels and into tissues. ICAM-1 is expressed by several cell types, typically on endothelial cells and cells of the immune system, and its expression can be up-regulated by various stimuli, including TNF- α , INF- γ , IL-1 and thrombin. It is a ligand for LFA-1 and Mac-1, serves as a receptor for rhinovirus, and is one of several receptors used by *Plasmodium falciparum*. ICAM-1 can exist as membrane-bound form (mICAM-1) and soluble form (sICAM-1). The sICAM-1 arises from alternative splicing and proteolysis of mICAM-1 and appears to be associated with leukocyte activation to produce LFA-1.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 2000 pg/bottle; lyophilized	2 bottles	
Detection antibody, biotinylated (100×) - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 1 - 30 mL/bottle. For mouse serum and plasma	2 bottles	
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernate and tissue homogenate	2 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

- 6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
- 6.4 Tissue Homogenates: The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1 x PBS to remove excess blood, Then add 1 mL of 1 x PBS per 100 mg tissue, homogenized and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 10000 x g at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

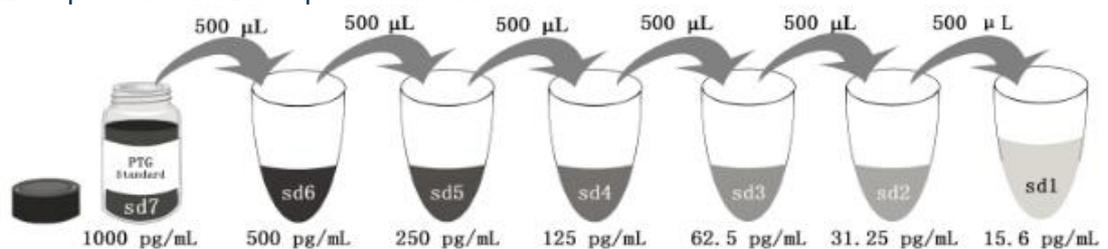
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:400 or 1:800 is recommended for mouse serum and plasma. Cell culture supernatant and tissue homogenate samples dilutions should be determined according to the customer's samples.

7.5 Standard Serial Dilution:

For mouse serum and plasma samples, add 2 mL Sample Diluent PT 1 in protein standard; For cell culture supernatant and tissue homogenate, add 2 mL Sample Diluent PT 1-ef in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL					
# µL of Sample Diluent PT 1 or PT 1-ef	2000 µL	500 µL					
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group, add 100 µL of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, it is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation 7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C.

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

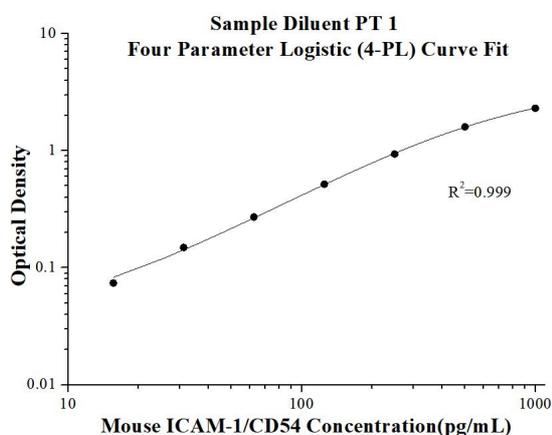
8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve-fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

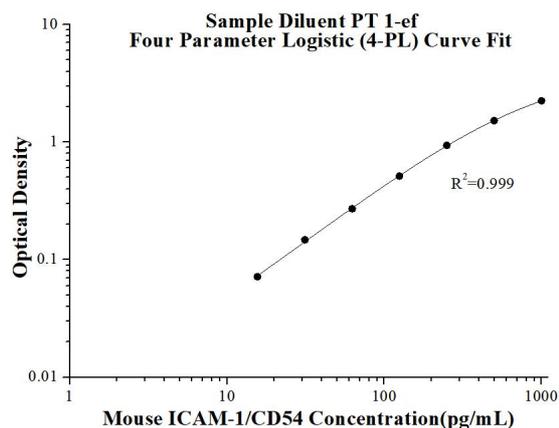
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.066 0.066	0.066	-
15.6	0.142 0.138	0.140	0.074
31.25	0.216 0.214	0.215	0.149
62.5	0.341 0.333	0.337	0.271
125	0.572 0.594	0.583	0.517
250	1.015 0.99	1.003	0.937
500	1.675 1.654	1.665	1.599
1000	2.406 2.338	2.372	2.306



(pg/mL)	O.D	Average	Corrected
0	0.063 0.063	0.063	-
15.6	0.133 0.136	0.135	0.072
31.25	0.211 0.21	0.211	0.148
62.5	0.332 0.336	0.334	0.271
125	0.578 0.573	0.576	0.513
250	1.009 0.993	1.001	0.938
500	1.584 1.586	1.585	1.522
1000	2.295 2.314	2.305	2.242

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	443.5	10.3	2.3	1	24	463.4	7.4	1.6
2	20	107.1	2.4	2.2	2	24	108.6	2.7	2.5
3	20	23.6	0.6	2.4	3	24	24.0	0.6	2.7

9.3 Recovery

The recovery of mouse ICAM-1 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:1,000	92	82-102
	1:2,000	96	85-116
Cell culture supernatant	1:50	86	79-94
	1:100	91	78-102
Tissue homogenate	1:600	84	79-89
	1:1,200	88	79-103

9.4 Sample values

Mouse serum - Mouse serum samples were evaluated for the presence of mouse ICAM-1/CD54 in this assay.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Mouse serum (n=16)	123	117-137

Cell culture supernatant - Lungs from one mouse were chopped into 1-2 mm pieces and seeded into approximately 30 mL of media containing RPMI, 10% fetal bovine serum, 50 μ M β -mercaptoethanol, and L-glutamine. An aliquot of the cell culture supernatant was removed for evaluation, assayed for levels of mouse ICAM-1/CD54, and measured 2.8 ng/mL.

Tissue Homogenate - Organs from 2 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized in 5-10 mL of PBS in a tissue homogenizer, and stored at $\leq -80^{\circ}\text{C}$ 5 min. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g to remove particulate. Homogenates from spleen and liver were assayed for mouse ICAM-1/CD54 and measured 89 ng/mL and 76 ng/mL, respectively.

9.5 Sensitivity

The minimum detectable dose of mouse ICAM-1/CD54 is 3.2 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The mouse serum were initially diluted 1:200. The cell culture supernatant was initially diluted 1:160. The tissue homogenate was initially diluted 1:10)

		Mouse serum (Sample Diluent PT 1)	Cell culture supernatant (Sample Diluent PT 1-ef)	Tissue homogenate (Sample Diluent PT 1-ef)
1:2	Average% of Expected	100	100	100
	Range (%)	-	-	-
1:4	Average% of Expected	98	90	107
	Range (%)	91-110	77-99	100-113
1:8	Average% of Expected	94	103	110
	Range (%)	80-115	84-117	99-119
1:16	Average% of Expected	95	106	115
	Range (%)	89-99	91-118	109-122

10. References

1. Lawson C, et al. (2009) *Pharmacol Rep.* 61(1):22-32.
2. Dustin ML, et al. (1986) *J Immunol.* 137(1):245-54.
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