

Rat ICAM-1/CD54 Sandwich ELISA Kit Datasheet

For the quantitative detection of rat ICAM-1/CD54 in rat serum and plasma samples.

General Information

Catalogue Number	KE20013
Product Name	Rat ICAM-1/CD54 Sandwich ELISA Kit
Species cross-reactivity	Rat
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	25464
SwissProt	Q00238

Kit Components & Storage

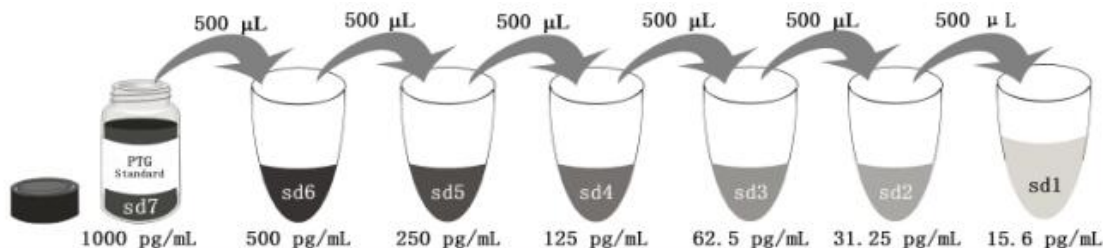
Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 1000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X), biotinylated - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	2 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ef is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE20013 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The ICAM-1/CD54 ELISA kit is to be used to detect and quantify protein levels of endogenous ICAM-1/CD54. The assay recognizes rat ICAM-1/CD54. An antibody specific for ICAM-1/CD54 has been pre-coated onto the microwells. The ICAM-1/CD54 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for ICAM-1/CD54 is added to detect the captured ICAM-1/CD54 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

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.

Sample Preparation

The sample may require proper dilution to fall within the range of the assay. 1:100 or 1:200 dilution is recommended for rat serum or plasma.

Safety Notes

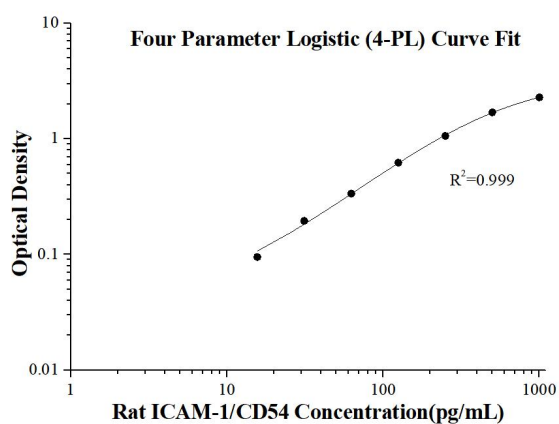
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

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.				

Example data

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	0.044 0.042	0.043	-
15.62	0.137 0.138	0.138	0.095
31.25	0.248 0.227	0.238	0.195
62.5	0.371 0.386	0.379	0.336
125	0.679 0.650	0.665	0.622
250	1.102 1.104	1.103	1.060
500	1.704 1.771	1.738	1.695
1000	2.321 2.331	2.326	2.283

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	547.4	13.9	2.5	1	24	546.0	12.5	2.3
2	20	124.0	3.3	2.6	2	24	124.4	2.7	2.2
3	20	29.0	0.9	3.2	3	24	27.6	0.7	2.4

Recovery

The recovery of rat ICAM-1/CD54 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Rat serum	1:150	84	79-89
	1:300	86	80-93

Sample Values

Sample Type	Mean (pg/mL)	Range (pg/mL)
Rat serum (n=16)	27,679	23,508-30,344

Sensitivity

The minimum detectable dose of rat ICAM-1/CD54 is 3.1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, three samples were spiked **Sample Diluent PT 1-ef** to produce samples with values within the dynamic range of the assay. (The serum were initially diluted 1:20)

		Rat serum
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	104
	Range (%)	102-108
1:8	Average% of Expected	110
	Range (%)	106-114
1:16	Average% of Expected	112
	Range (%)	102-121

References

1. Lawson C, et al. ICAM-1 signaling in endothelial cells. *Pharmacol Rep.* 61(1):22-32 (2009).
2. Dustin ML, et al. Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J Immunol.* 137(1):245-54 (1986).
3. Marlin SD, et al. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell.* 51(5):813-9 (1987).
4. Staunton DE, et al. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell.* 56(5):849-53 (1989).
5. Berendt AR, et al. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature.* 341(6237):57-9 (1989).
6. Ramos TN, et al. ICAM-1: isoforms and phenotypes. *J Immunol.* 192(10):4469-4474 (2014).