Quantikine® ELISA

Rat sICAM-1/CD54 Immunoassay

Catalog Number RIC100

For the quantitative determination of rat soluble Intercellular Adhesion Molecule-1 (sICAM-1) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Rat Intercellular Adhesion Molecule 1 (ICAM-1), also known as CD54, is an 80-110 kDa, type I transmembrane glycoprotein that is expressed on a variety of cell types (1-3). The molecule is 545 amino acids (aa) in length and contains a 27 aa signal sequence, a 466 aa extracellular region, a 25 aa transmembrane segment, and a 27 aa cytoplasmic domain (3). The extracellular region contains five Ig-like domains and eleven potential N-linked glycosylation sites. The first N-terminal Ig domain (D1) binds LFA-1 (lymphocyte function associated antigen type 1), while the third domain (D3) binds Mac-1, also known as integrin alpha-M (1). The cytoplasmic domain, while short, can both transduce intracellular signals (via MAP kinase) (4, 5) and interact with the cytoskeleton (4). While membrane ICAM-1 exists as a dimer (6, 7), monomeric ICAM-1 is competent to bind LFA-1 as well (8). Soluble forms of ICAM-1 are generated via proteolytic processing, reportedly by MMP-9 (9) and elastase (10). Soluble, dimeric ICAM-1 circulates and binds LFA-1 with high avidity (11). In mouse, a number of ICAM-1 alternate splice forms lacking various Ig-domains (12) exist, suggesting the possibility of multiple truncated forms of proteolytically-generated circulating ICAM-1. As the mature rat ICAM-1 shows 77% aa sequence identity to mouse ICAM-1 (13), a similar splicing pattern is likely (14). Mature rat ICAM-1 is 52%, 53%, and 49% identical at the aa level to human (15), canine (16), and porcine (17) ICAM-1, respectively.

Cells known to express ICAM-1 include smooth muscle cells, keratinocytes, endothelial cells, fibroblasts, bronchial epithelial cells (18), memory T cells, B cells, plasma cells, monocytes, macrophages, CFU-E, CFU-GM, activated eosinophils and neutrophils (19), Schwann cells, Sertoli cells, melanocytes, and dendritic cells (2). There are at least four known ligands (or co-receptors) for ICAM-1. Two of these are the integrins LFA-1/$\alpha_L\beta_2$ (CD11a/CD18) and Mac-1/$\alpha_M\beta_2$ (CD11b/CD18) (1, 2). The β2 integrins are restricted to leukocytes with lymphocytes preferentially expressing LFA-1, and NK cells, neutrophils, and monocytes expressing both LFA-1 and Mac-1 (1). The third ligand is CD43/sialophorin (20) and the fourth is fibrinogen (5).

ICAM-1 is a generally inducible transmembrane molecule that plays a role in cell migration, antigen presentation, and leukocyte activation (1, 2, 21). When ligated to its principal co-receptor LFA-1, ICAM-1 can have up to three effects. First, it can provide anchorage to cells expressing LFA-1. During inflammation, endothelial cells (EC) expressing ICAM-1 mediate the adhesion and trans-endothelial migration of circulating LFA-1$^+$ leukocytes (19, 21). In addition, APC ICAM-1 and T cell LFA-1 can create an adhesive interface that prolongs effective antigen presentation under antigen-limiting conditions (22, 23). Second, ICAM-1 ligation can induce singular effects on ICAM-1 expressing cells. EC ICAM-1 transmits signals that activate intracellular Rho, leading to cytoskeletal rearrangement and endothelial cell contraction (4). On T cells, ICAM-1 activation, in conjunction with CD3 engagement, induces naive T cell proliferation and Th1 (IL-2/IFN-γ) cytokine release (24). Third, ICAM-1 as a "passive ligand" can promote specific LFA-1 mediated activities. These include the generation of cytokine-secreting inflammatory effector CD4$^+$ T cells (25), and the trans-activation (modulation) of same-cell tyrosine kinase growth factor receptors following integrin ligation and clustering (26).

The Quantikine Rat sICAM-1/CD54 Immunoassay is a 4.5 hour solid phase ELISA designed to measure rat sICAM-1 levels in cell culture supernates, serum, and plasma. It contains NS0-expressed, recombinant rat sICAM-1 and antibodies raised against the recombinant protein. Results obtained for naturally occurring rat sICAM-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values of natural rat sICAM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat sICAM-1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any rat sICAM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat sICAM-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of rat sICAM-1 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Rat sICAM-1 Microplate	892415	One 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat sICAM-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Rat sICAM-1 Conjugate	892416	12 mL of a polyclonal antibody against rat sICAM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Rat sICAM-1 Standard	892417	10 ng of recombinant rat sICAM-1 in a buffered protein base with preservatives; lyophilized.	
Rat sICAM-1 Control	892418	1 vial of recombinant rat sICAM-1 in a buffered protein base with preservatives; lyophilized. The concentration range of recombinant rat sICAM-1 after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Heparin and citrate plasma have not been validated for use in this assay.*

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require at least a 50-fold dilution prior to assay. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-26 (1X).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Rat sICAM-1 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

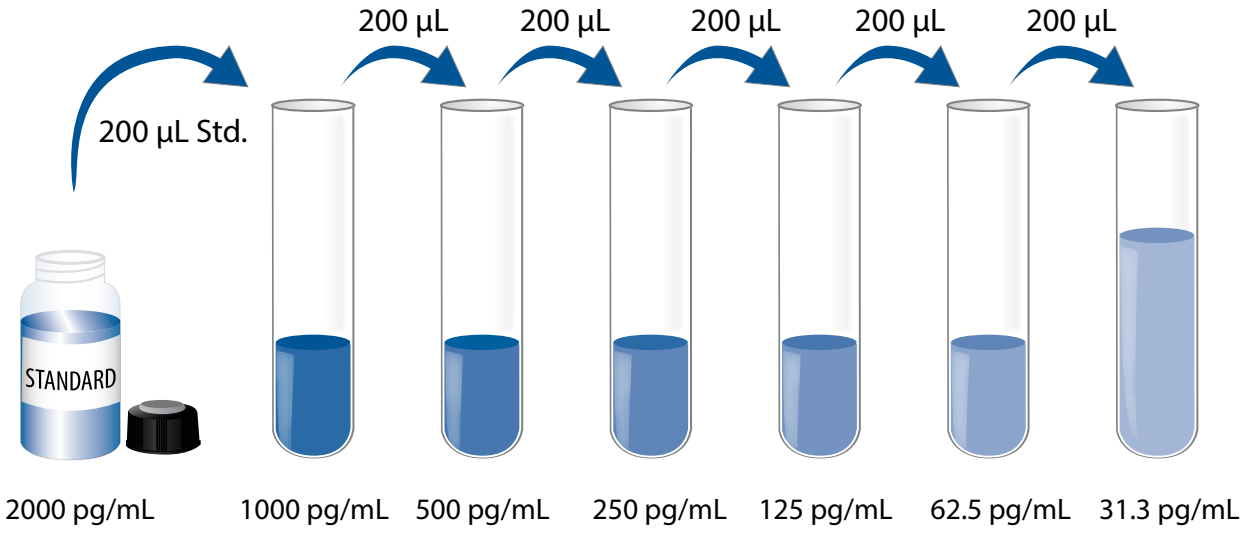
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (1X) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (1X).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Rat sICAM-1 Standard - Reconstitute the Rat sICAM-1 Standard with 5.0 mL of Calibrator Diluent RD5-26 (1X). Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-26 (1X) into each tube. Use the standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Rat sICAM-1 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (1X) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, Control, and samples be assayed in duplicate.

1. Prepare reagents, standard dilutions, Control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Rat sICAM-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Serum and plasma samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

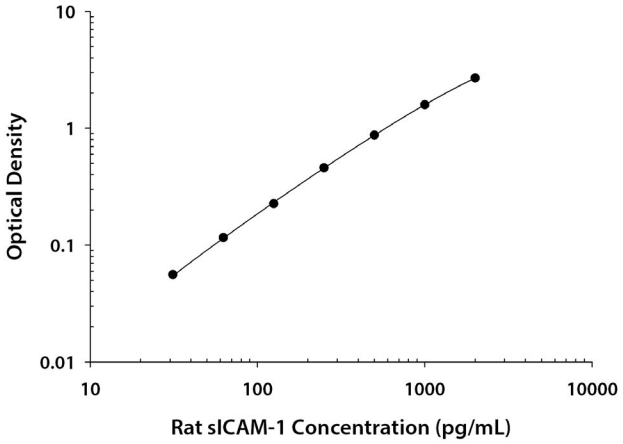
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the rat sICAM-1-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.068 0.072	0.070	—
31.3	0.125 0.126	0.126	0.056
62.5	0.183 0.188	0.186	0.116
125	0.295 0.297	0.296	0.226
250	0.527 0.531	0.529	0.459
500	0.923 0.963	0.943	0.873
1000	1.627 1.686	1.656	1.586
2000	2.733 2.783	2.758	2.688

PRECISION

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

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

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	47	39	47
Mean (pg/mL)	87	271	617	80	307	560
Standard deviation	4.3	13	29	7.6	19	35
CV (%)	4.9	4.8	4.7	9.5	6.2	6.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernate (n=8)	99	88-104%
Serum* (n=6)	103	83-119%
EDTA plasma* (n=5)	103	91-117%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of rat ICAM-1 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=6)	Serum* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	95	101	98
	Range (%)	86-102	94-109	88-104
1:4	Average % of Expected	93	102	98
	Range (%)	89-96	96-109	88-109
1:8	Average % of Expected	93	100	94
	Range (%)	86-99	94-112	87-106
1:16	Average % of Expected	93	100	94
	Range (%)	88-102	95-110	85-104

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of rat ICAM-1 ranged from 1.2-4.1 pg/mL. The mean MDD was 2.0 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant rat ICAM-1/Fc Chimera produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma -Samples were evaluated for the presence of rat ICAM-1 in this assay.

Sample	Mean (pg/mL)	Range (pg/mL)
Serum (n=20)	31,233	17,375-49,170
EDTA plasma (n=20)	24,569	11,160-39,570

Cell Culture Supernates:
Rat heart, kidney, and lung tissues were cultured for 4 days in DMEM supplemented with 10% fetal calf serum and stimulated with 2.5 ng/mL LPS. Aliquots of the cell culture supernates were removed, assayed for rat ICAM-1, and measured 1226 pg/mL, 5500 pg/mL, and 4000 pg/mL, respectively.

Rat spleen was cultured for 4 days in DMEM supplemented with 10% fetal calf serum and stimulated with 50 ng/mL PMA. An aliquot of the cell culture supernate was removed, assayed for rat ICAM-1, and measured 2303 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat ICAM-1.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rat ICAM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:
E-Selectin

Recombinant mouse:
ICAM-1
ICAM-2
E-Selectin
L-Selectin
P-Selectin
VCAM-1

Recombinant human:
ICAM-1
ICAM-2
ICAM-3

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