Quantikine® ELISA

Mouse ICAM-1/CD54 Immunoassay

Catalog Number MIC100

For the quantitative determination of mouse soluble Intercellular Adhesion Molecule-1 (ICAM-1) concentrations in cell culture supernates, tissue homogenates, 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

Intercellular Adhesion Molecule-1 (ICAM-1) is an inducible transmembrane molecule that plays a role in cell migration, antigen presentation and leukocyte activation (1-3). When ligated to its principal counter-receptor, LFA-1, ICAM-1 can play three different roles. First, it provides anchorage to cells expressing LFA-1. During inflammation, endothelial cells (EC) expressing ICAM-1 mediate the adhesion and transendothelial migration of circulating LFA-1$^+$ leukocytes (3, 4). In addition, antigen presenting cell (APC) ICAM-1 and T cell LFA-1 can create an adhesive interface that prolongs effective antigen presentation under limiting antigen concentrations (5, 6). Second, ICAM-1 is a receptor that transduces signals on ICAM-1 expressing cells. EC ICAM-1 transmits signals that activate intracellular Rho, leading to cytoskeleton rearrangement and EC contraction (7). On T cells, ICAM-1 activation, in conjunction with CD3 engagement, induces naive T cell proliferation and Th1 (IL-2/IFN-γ) cytokine release (8). Third, ICAM-1 is a ligand that can activate specific LFA-1 mediated activities. These include the generation of cytokine-secreting inflammatory effector CD4$^+$ T cells (9), and the trans-activation (modulation) of tyrosine kinase growth factor receptors following integrin ligation and clustering (10).

Mouse ICAM-1 is an 80-110 kDa, type I transmembrane glycoprotein that is expressed on a variety of cell types (1, 2, 11, 12). The molecule is 537 amino acids (aa) in length and contains a 27 aa signal sequence, a 458 aa extracellular region, a 24 aa transmembrane segment, and a 28 aa cytoplasmic domain (11, 12). The extracellular region contains five Ig-like domains and eleven potential N-linked glycosylation sites, many of which are utilized. The first, N-terminal Ig domain (D1) binds LFA-1, while the third domain (D3) binds Mac-1 (1). Notably, glycosylation on the third domain regulates Mac-1 binding (13). The cytoplasmic domain, while short, is considered to both transduce intracellular signals (via MAP kinase) (7, 14) and interact with the cell cytoskeleton (7). Membrane ICAM-1 exists as a dimer and will form multimers via D1 interactions (15, 16). Monomeric ICAM-1 is competent to bind LFA-1 (17). Soluble, dimeric ICAM-1 does circulate and binds LFA-1 with high avidity (18). Soluble forms are generated via proteolytic processing, reportedly through MMP-9 (19) and elastase (20). In mice, there are a number of ICAM-1 alternate splice forms that lack combinations of various Ig-domains (21). This suggests the possibility of multiple truncated forms of proteolytically-generated circulating ICAM-1. Mature mouse ICAM-1 shares 77%, 53%, 56%, and 52% sequence identity to rat (22), human (23), canine (24) and porcine (25) ICAM-1, respectively. Cells known to express ICAM-1 include smooth muscle cells, keratinocytes, endothelial cells, fibroblasts, bronchial epithelial cells (26), memory T cells, B cells, plasma cells, monocytes, macrophages, CFU-E, CFU-GM, activated eosinophils, neutrophils (4), Schwann cells, Sertoli cells, melanocytes, and dendritic cells (2).

ICAM-1 binds at least four known ligands/counter-receptors. Two of these are the integrins LFA-1/αLβ2 (CD11a/CD18) and Mac-1/αMβ2 (CD11b/CD18) (1, 2). The β_2 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). A third ligand is CD43/sialophorin (27) and the fourth ligand is fibrinogen (14).

The Quantikine soluble Mouse ICAM-1/CD54 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse sICAM-1 levels in cell culture supernates, tissue homogenates, serum, and plasma. It contains NS0-expressed recombinant mouse ICAM-1 and antibodies raised against the recombinant protein. Results obtained for naturally occurring mouse ICAM-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 mouse ICAM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse ICAM-1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse ICAM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse ICAM-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 mouse ICAM-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.
- For best results, pipette reagents and samples into the center of each well.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between steps may improve assay precision.
- 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
Mouse ICAM-1 Microplate	892480	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse ICAM-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.*
Mouse ICAM-1 Standard	892482	Recombinant mouse ICAM-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Mouse ICAM-1 Control	892483	1 vial of recombinant mouse ICAM-1 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse ICAM-1 Conjugate	892481	12 mL of a polyclonal antibody against mouse ICAM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
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 base with preservatives. *Used diluted 1:4 in this assay.*	
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.
- **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 a preservative 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.

Tissue Homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, spleen or liver tissue from one adult female mouse was rinsed with PBS to remove excess blood, homogenized in 5 mL of 1X PBS, and stored at ≤ -20 °C overnight. Homogenates were centrifuged for 5 minutes at 2000 x g.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Use polypropylene tubes.

Tissue homogenate samples require a 10-fold dilution prior to assay. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calbrator Diluent RD5-26 (diluted 1:4)*.

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 (diluted 1:4).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

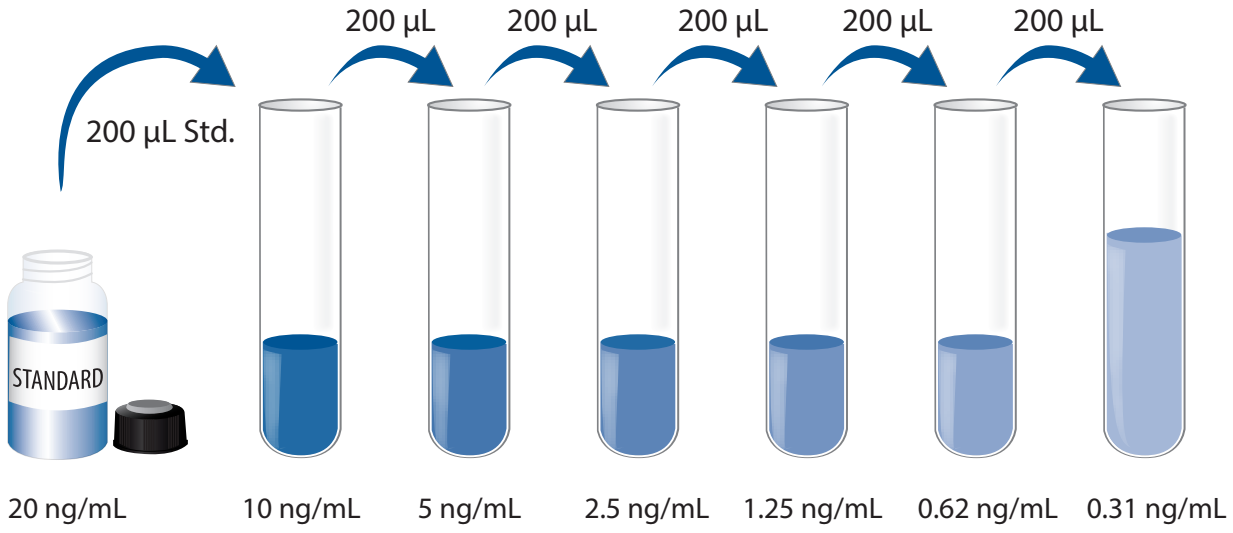
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (diluted 1:4) - 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 (diluted 1:4).

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.

Mouse ICAM-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse ICAM-1 Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 20 ng/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 (diluted 1:4) 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 Mouse ICAM-1 Standard (20 ng/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/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.
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 Mouse ICAM-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
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. **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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

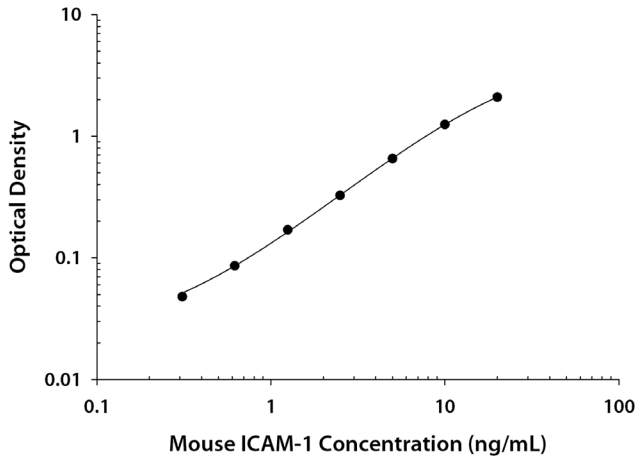
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse ICAM-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.



(ng/mL)	O.D.	Average	Corrected
0	0.025 0.026	0.026	—
0.31	0.073 0.074	0.074	0.048
0.62	0.106 0.117	0.112	0.086
1.25	0.196 0.196	0.196	0.170
2.5	0.348 0.355	0.352	0.326
5	0.676 0.686	0.681	0.655
10	1.250 1.297	1.274	1.248
20	2.099 2.141	2.120	2.094

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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.99	2.61	8.34	1.23	2.84	7.87
Standard deviation	0.10	0.15	0.76	0.09	0.16	0.60
CV (%)	10.1	5.7	9.1	7.3	5.6	7.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	104	98-113%
Serum* (n=6)	95	80-119%
EDTA plasma* (n=7)	98	83-113%
Heparin plasma* (n=7)	104	85-120%

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

LINEARITY

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

		Cell culture supernates (n=5)	Tissue Homogenates* (n=2)	Serum* (n=5)	EDTA plasma* (n=5)	Heparin plasma* (n=6)
1:2	Average % of Expected	97	110	104	100	101
	Range (%)	92-101	106-114	99-112	95-108	93-104
1:4	Average % of Expected	94	116	104	107	102
	Range (%)	91-102	113-118	88-108	99-114	85-109
1:8	Average % of Expected	96	115	108	102	105
	Range (%)	93-100	114-115	103-115	98-110	80-110
1:16	Average % of Expected	98	113	115	104	104
	Range (%)	94-103	111-115	112-118	92-114	80-119

*Samples were diluted prior to assay.

SENSITIVITY

Thirteen assays were evaluated and the minimum detectable dose (MDD) of mouse ICAM-1 ranged from 0.017-0.057 ng/mL. The mean MDD was 0.029 ng/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 mouse ICAM-1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of mouse ICAM-1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=20)	389	156-518
EDTA Plasma (n=20)	392	302-596
Heparin Plasma (n=20)	373	311-422

Cell Culture Supernates:
J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM containing 10% fetal bovine serum and stimulated with 100 ng/mL of recombinant mouse IFN-γ and 1.0 µg/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse ICAM-1, and measured 11 ng/mL.

Two mouse lungs (1-2 mm pieces in 40 mL of medium) were cultured for 7 days in RPMI supplemented with 10% fetal bovine serum. An aliquot of conditioned media was removed, assayed for mouse ICAM-1, and measured 91 ng/mL.

Tissue Homogenates - The homogenates from the spleen and liver tissues, prepared as described in the Sample Collection & Storage section, were assayed for mouse ICAM-1 and measured 142 ng/mL and 86 ng/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant mouse 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 mouse ICAM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

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

Recombinant rat:
ICAM-1

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

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