Quantikine®

Mouse sL-Selectin Immunoassay

Catalog Number MLS00

For the quantitative determination of mouse soluble L-Selectin (sL-Selectin) concentrations in cell culture supernates, mouse serum, and EDTA plasma.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

TABLE OF CONTENTS

Contents	Page
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	3
LIMITATIONS OF THE PROCEDURE	3
PRECAUTION	3
TECHNICAL HINTS	3
MATERIALS PROVIDED	4
STORAGE	4
OTHER SUPPLIES REQUIRED	5
SAMPLE COLLECTION AND STORAGE	5
SAMPLE PREPARATION	5
REAGENT PREPARATION	6
ASSAY PROCEDURE	7
PROCEDURE SUMMARY AND CHECKLIST	8
CALCULATION OF RESULTS	9
TYPICAL DATA	9
PRECISION	10
RECOVERY	10
LINEARITY	11
SENSITIVITY	11
CALIBRATION	11
SAMPLE VALUES	12
SPECIFICITY	13
REFERENCES	14
PLATE LAYOUT	15

MANUFACTURED AND DISTRIBUTED BY:

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

TELEPHONE: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems China Co. Ltd.
24A1 Hua Min Empire Plaza
726 West Yan An Road
Shanghai PRC 200050

TELEPHONE: +86 (21) 52380373
FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

L-Selectin (also known as CD62L, LAM-1, LECAM-1, Ly22, gp90, MEL-14 and Leu-8) is a member of a small family of transmembrane leukocyte adhesion molecules that share common structural motifs (1 - 3). Members of this family, which also include E- and P-Selectin, show little sequence identity but all contain a lectin-binding domain, an EGF-like motif, and multiple short consensus repeats (SCRs). Mature mouse L-selectin is a constitutively-expressed 90 kDa, 334 amino acid (aa) type I transmembrane glycoprotein. It has a 295 aa extracellular region that contains a 116 aa lectin-binding domain, a 34 aa EGF-like domain, and two identical 62 aa SCR motifs. This is followed by a 22 aa transmembrane domain and a 17 aa cytoplasmic tail that can activate signal transduction pathways (4 - 8). Polymorphisms exist for L-selectin in inbred mouse strains within the EGF-like region. The monoclonal antibody that defines the Ly22 cell surface marker recognizes a polypeptide determinant of the EGF-like domain of an $Ly22^+$ mouse strain (C3H) that differs from an $Ly22^-$ mouse strain (SJL) by a single amino acid substitution (4). Mouse L-Selectin shares 77% and 85% aa identity with human and rat L-Selectin, respectively. The highest similarity is in the lectin and EGF domains of the proteins (9 - 11). Cells known to express L-Selectin include neutrophils, monocytes, eosinophils, basophils, B cells, $\gamma\delta$ T cells and naive $\alpha\beta$ T cells (8, 12, 13).

L-Selectin on lymphocytes interacts with its ligand(s) on endothelial cells of high endothelial venules, where it promotes lymphocyte homing to the lymph nodes (1, 2, 13 - 15). L-Selectin is also involved with leukocyte recruitment into areas of inflammation, where it binds to L-Selectin ligands on leukocytes that are already bound to endothelium. This creates leukocytic strata or layers of cells that ultimately find their way to the endothelium and into the tissues (13, 16 - 18). Although selectins are often thought of as initial points of adhesion for circulating leukocytes, L-Selectin has been found to be rapidly proteolytically cleaved following engagement. This allows transient anchoring and insures continued physiologic leukocyte rolling at typical flow velocities (19). L-Selectin binds to a number of ligands, including a sulfated sialyl Lewis-x (sLe^x), 6-sulfo sLe^x (sialic acid α 2$\rightarrow$3 galactose β1$\rightarrow$4 [fucose α1$\rightarrow$3 (sulfo$\rightarrow$6)] N-acetylglucosamine) that is present on CD34, GlyCAM-1, Spg200 (15, 20, 21), type IV collagen (22), PCLP (or podocalyxin-like protein) (23), MAdCAM-1 (24), PSGL-1 (25), cutaneous lymphocyte antigen (CLA) (26), and chondroitin sulfate/heparin sulfate (27). E-Selectin is a ligand for human but not mouse L-Selectin (28).

The 68 kDa soluble form of L-Selectin is known to exist in circulation at high levels, and this is reported to arise from proteolytic cleavage by ADAM17. Leukocyte L-Selectin molecules are rapidly shed during physiologic rolling, and this shedding is suggested to limit white cell aggregation and/or unintended leukocyte binding to normal endothelium (29 - 32).

The Quantikine Mouse soluble L-Selectin (sL-Selectin) Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse sL-Selectin levels in cell culture supernates, serum, and EDTA plasma. It contains NS0-expressed recombinant mouse L-Selectin and antibodies raised against the recombinant protein. Results obtained for naturally occurring mouse sL-Selectin 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 sL-Selectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse sL-Selectin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse sL-Selectin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse sL-Selectin 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 sL-Selectin 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 operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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.
- 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

Mouse sL-Selectin Microplate (Part 890982) - One 96 well polystyrene microplate (12 strips of 8 wells) coated with a rat monoclonal antibody specific for mouse sL-Selectin.

Mouse sL-Selectin Conjugate (Part 890983) - 12.5 mL of a monoclonal antibody against mouse sL-Selectin conjugated to horseradish peroxidase with preservatives.

Mouse sL-Selectin Standard (Part 890984) - 3 vials (80 ng/vial) of recombinant mouse sL-Selectin in a buffered protein base with preservatives; lyophilized.

Mouse sL-Selectin Control (Part 890286) - 3 vials of recombinant mouse sL-Selectin in a buffered protein base with preservatives; lyophilized. The concentration range of mouse sL-Selectin 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 (Part 895215) - 12.5 mL of a buffered protein solution with preservatives.

Calibrator Diluent RD5-26 Concentrate (Part 895525) - 21 mL of a 4-fold concentrated buffered protein solution with preservatives.

Wash Buffer Concentrate (Part 895024) - 50 mL of a 25-fold concentrated solution of a buffered surfactant with preservative.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895174) - 23 mL of a diluted hydrochloric acid solution.

Plate Covers (Part 640197) - 4 adhesive plate sealers.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use beyond kit expiration date.</td></tr>
<tr><td rowspan="11">Opened/ Reconstituted Reagents</td><td>Mouse sL-Selectin Conjugate</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Diluted Wash Buffer</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent RD5-26</td></tr>
<tr><td>Assay Diluent RD1-21</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>mouse sL-Selectin Standard (40 ng/mL)</td><td rowspan="2">Use within 8 hours of reconstitution. Use a fresh standard and control for each assay.</td></tr>
<tr><td>mouse sL-Selectin Control</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

*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 1000 mL graduated cylinders.
- **Polypropylene** test tubes for dilution.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and 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 or overnight at 2 - 8° C 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 as an anticoagulant. Centrifuge at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Use polypropylene tubes. Mouse serum and plasma samples generally require at least a 50-fold dilution into Calibrator Diluent RD5-26 (1X) prior to assay. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-26 (1X). Mix well.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse sL-Selectin Kit Control - Reconstitute the Kit Control with 1.0 mL of deionized or distilled water. Assay the Control undiluted.

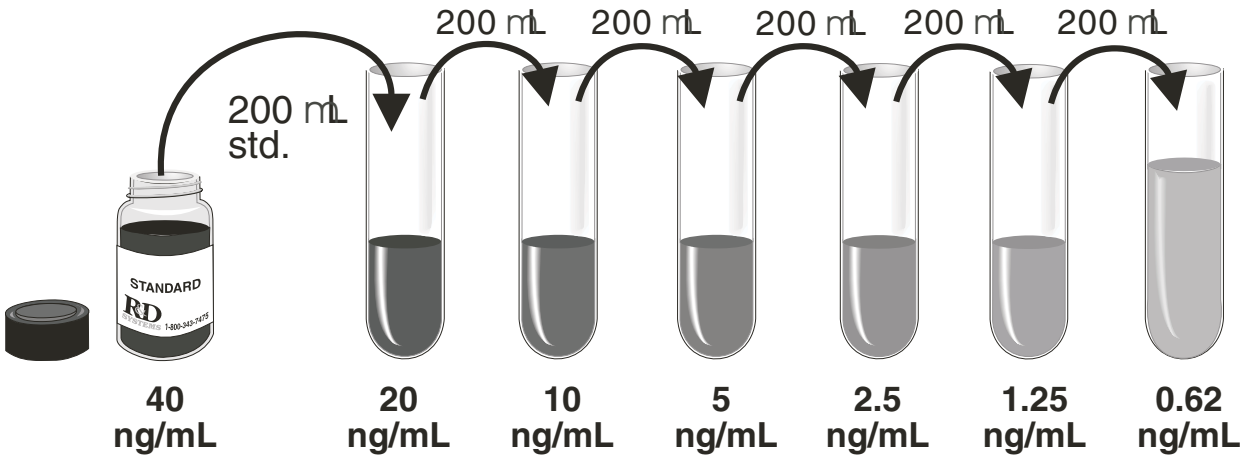
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 625 mL of Wash Buffer.

Calibrator Diluent RD5-26 (1X) - Dilute 20 mL of Calibrator Diluent RD5-26 Concentrate into 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.

Mouse sL-Selectin Standard - Reconstitute the mouse sL-Selectin Standard with 2.0 mL of Calibrator Diluent RD5-26 (1X). Do not substitute other diluents. This reconstitution produces a stock solution of 40 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 (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 mouse sL-Selectin Standard serves as the high standard (40 ng/mL). Calibrator Diluent RD5-26 (1X) 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 samples, standards, and control be assayed in duplicate.

1. Prepare reagents, standard dilutions, and samples as directed by 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. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.

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 sL-Selectin 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.

*Mouse serum and plasma samples require at least a 50-fold dilution. See Sample Preparation.

PROCEDURE SUMMARY AND CHECKLIST

1. ☐ Bring all reagents to room temperature.
☐ Prepare reagents and samples as instructed.
☐ Return unused components to storage temperature as indicated in the instructions.

2. ☐ Add 50 µL Assay Diluent to each well.

3. ☐ Add 50 µL Standard, Control, or sample* to each well.
☐ Tap plate gently for one minute.
☐ Cover the plate and incubate 2 hours at room temperature.

4. ☐ Aspirate and wash each well five times.

5. ☐ Add 100 µL Conjugate to each well.
☐ Cover the plate and incubate 2 hours at room temperature.

6. ☐ Aspirate and wash each well five times.

7. ☐ Add 100 µL Substrate Solution to each well.
Incubate 30 minutes at room temperature.
Protect from light.

8. ☐ Add 100 µL Stop Solution to each well.

9. ☐ Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

*Mouse serum and plasma samples require at least a 50-fold dilution. See Sample Preparation.

CALCULATION OF RESULTS

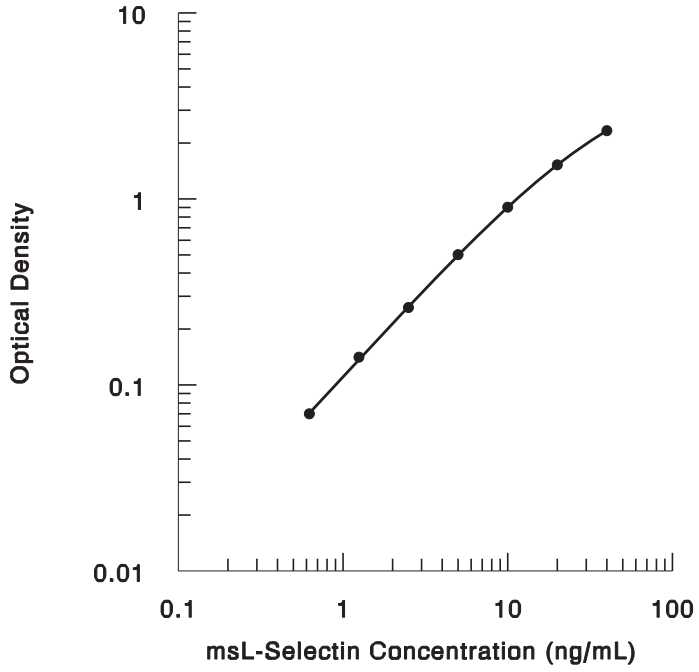
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 sL-Selectin 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.

Because mouse serum and plasma samples have been diluted prior to assay, 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	0.008 0.008	0.008	—
0.62	0.076 0.079	0.078	0.070
1.25	0.145 0.152	0.148	0.140
2.5	0.259 0.278	0.268	0.260
5	0.507 0.512	0.510	0.502
10	0.893 0.928	0.910	0.902
20	1.510 1.560	1.535	1.527
40	2.300 2.370	2.335	2.327

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 assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	1.43	6.95	18.2	1.63	7.43	19.3
Standard deviation	0.07	0.50	1.5	0.10	0.50	1.5
CV (%)	4.9	7.2	8.2	6.1	6.7	7.8

RECOVERY

The recovery of mouse sL-Selectin spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Mouse cell culture supernate (n = 6)	94	83 - 109%
Mouse serum* (n = 10)	93	80 - 116%
Mouse EDTA plasma* (n = 6)	93	80 - 119%

*Mouse serum and plasma samples were diluted 100-fold prior to assay.

LINEARITY

To assess the linearity of the assay, six samples containing and/or spiked with high concentrations of mouse sL-Selectin in each matrix were diluted with Calibrator Diluent RD5-26 (1X) and then assayed.

		Cell culture supernate	Serum*	EDTA plasma*
1:2	Average % of Expected	103	99	96
	Range (%)	98 - 106	98 - 102	92 - 103
1:4	Average % of Expected	102	96	99
	Range (%)	96 - 106	93 - 102	94 - 107
1:8	Average % of Expected	104	94	94
	Range (%)	99 - 110	89 - 100	90 - 102
1:16	Average % of Expected	104	92	93
	Range (%)	99 - 107	84 - 95	82 - 104

*Mouse serum and plasma samples were diluted 50-fold prior to assay.

SENSITIVITY

Two assays were evaluated and the minimum detectable dose (MDD) of mouse sL-Selectin ranged from 29 - 34 pg/mL. The mean MDD was 31.5 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 mouse L-Selectin/Fc Chimera produced at R&D Systems. This mature recombinant mouse protein contains amino acid residues Trp 39 - Asu 332 (extracellular domain) of mouse L-Selectin fused to the carboxy-terminal six histidine-tagged Fc region of human IgG_1 via a polypeptide linker. Recombinant mouse L-Selectin/Fc Chimera is a disulfide-linked homodimeric protein. Each monomer has a calculated molecular mass of approximately 60.5 kDa. As a result of glycosylation, each monomer of the recombinant mouse L-Selectin/Fc Chimera migrates as an approximately 150 kDa protein in SDS-PAGE under reducing conditions.

The protein concentration of the recombinant mouse L-Selectin was determined by the method of Bradford (33) using purified bovine serum albumin as a standard.

SAMPLE VALUES

Serum/Plasma - Twenty individual mouse serum and plasma samples were evaluated for detectable levels of mouse sL-Selectin in this assay.

Cell Culture Supernates -
Lungs from two mice were cultured for 6 days in 40 mL of RPMI supplemented with 10% fetal calf serum and evaluated for detectable levels of mouse sL-Selectin in this assay.

Mouse WEHI-3 cells were cultured for 3 days in RPMI supplemented with 10% fetal calf serum and evaluated for detectable levels of mouse sL-Selectin in this assay.

Sample	Mean (ng/mL)	Range (ng/mL)
Mouse serum	966	603 - 1260
Mouse plasma (EDTA)	601	396 - 808
Mouse lung conditioned media	4	——
Mouse WEHI-3 conditioned media	15	——

Note: *Normal rat and porcine serum samples were also evaluated and no sL-Selectin was detected.*

SPECIFICITY

This assay recognizes both recombinant and natural mouse sL-Selectin. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-26 (1X) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse sL-Selectin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
Eotaxin
sE-selectin
Fas Ligand
Flt-3 Ligand
G-CSF
GM-CSF
sICAM-2
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 sR
IL-12 p40
IL-12 p70
IL-13
IL-17
IL-18
JE/MCP-1
KC
Leptin
LIF
MARC
MCP-5
M-CSF
MIP-1α
MIP-1β
MIP-1γ
MIP-2
OPG
OSM
P*l*GF-2
sP-Selectin
RANK
RANK Ligand
RANTES
SCF
TARC
TNF-α
sTNF RI
sTNF RII
Tpo
VEGF
VEGF RI

Some cross-reactivity was observed with the following:

Factor	Concentration Tested (ng/mL)	Observed Value (ng/mL)	% Cross-reactivity
rhsL-Selectin	62	0.1	0.2
rmVCAM-1	1000	0.9	0.1

REFERENCES

1. Carlos, T.M. and J.M. Harlan (1994) Blood **84**:2068.
2. Lowe, J.B. (1997) Kidney Intl. **51**:1418.
3. Malhotra, R. and M.I. Bird (1997) BioEssays **19**:919.
4. Siegelman, M.H. *et al*. (1990) Cell **61**:611.
5. Siegelman, M.H. *et al*. (1989) Science **243**:1165.
6. Lasky, L.A. *et al.* (1989) Cell **56**:1045.
7. Steeber, D.A. *et al*. (1997) J. Immunol. **159**:952.
8. Hwang, S.T. *et al*. (1996) J. Exp. Med. **184**:1343.
9. Bowen, B.R. *et al*. (1989) J. Cell Biol. **109**:421.
10. Tedder, T.F. *et al*. (1989) J. Exp. Med. **170**:123.
11. Watanabe, T. *et al*. (1992) Biochim. Biophys. Acta **1131**:321.
12. Kansas, G.S. (1992) APMIS **100**:287.
13. Bird, M.I. *et al*. (1997) Biochem. Sco. Trans. **25**:1199.
14. Catalina, M.D. *et al.* (1996) J. Exp. Med. **184**:2341.
15. Rosen, S.D. and C.R. Bertozzi (1996) Curr. Biol. **6**:261.
16. Alon, R. *et al*. (1996) J. Cell Biol. **135**:849.
17. Fuhlbrigge, R.C. *et al.* (1996) J. Cell. Biol. **135**:837.
18. Patel, K.D. and R.P. McEver (1997) J. Immunol. **159**:4555.
19. Hafezi-Moghadam, A. and K. Ley (1999) J. Exp. Med. **189**:939.
20. Hemmerich, S. *et al*. (1994) J. Exp. Med. **180**:2219.
21. Hemmerich, S. *et al*. (1995) J. Biol. Chem. **270**:12035.
22. Iwabuchi, K. *et al*. (1996) Blood **87**:365.
23. Sassetti, C. *et al*. (1998) J. Exp. Med. **187**:1965.
24. Berg, E.L. *et al*. (1993) Nature **366**:695.
25. Guyer, D.A. *et al*. (1996) Blood **88**:2415.
26. Tu, L. *et al*. (1999) J. Exp. Med. **189**:241.
27. Li, Y-F. *et al*. (1999) FEBS Lett. **444**:201.
28. Zollner, O. *et al*. (1997) J. Cell Biol. **136**:707.
29. Migaki, G.I. *et al*. (1995) J. Exp. Med. **182**:549.
30. Condon, T.P. *et al*. (2001) Antisense Nucleic Acid Drug Dev. **11**:107.
31. Walcheck, B. *et al.* (1996) Nature **380**:720.
32. Schleiffenbaum, B. *et al*. (1992) J. Cell Biol. **119**:229.
33. Bradford, M.M (1976) Anal. Biochem. **72**:248.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.