Human sL-Selectin/CD62L Immunoassay

Catalog Number BBE4B
SBBE4B
PBBE4B

For the quantitative determination of human sL-Selectin in cell culture supernates, serum, and plasma.

Note: The part number of the Wash Buffer in this kit has changed, however, the formulation has not changed. Please read this package insert in its entirety before using this product.

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

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Human L-Selectin (leukocyte selectin, CD62L, LAM-1, LECAM-1, LECCAM-1, TQ1, Leu-8, DREG, lymph node homing receptor, MEL-14 antigen) is a cell surface glycoprotein expressed constitutively on a wide variety of leukocytes (1-3). A member of the Selectin family of cell surface molecules, L-Selectin consists of an NH_2-terminal calcium-dependent lectin domain, an EGF-like domain, two CRP-like domains, a transmembrane sequence and a short cytoplasmic domain (1, 4, 5). Human and mouse L-Selectin show the same domain structure and are 77% identical at the amino acid level and 79% identical at the nucleotide level (4, 6). Two forms of L-Selectin have been reported, apparently arising as a result of post-translational modifications. The lymphocyte form shows an apparent molecular weight of 74 kDa, while the neutrophil form is 90-100 kDa (7).

L-Selectin plays a role in the migration of lymphocytes into peripheral lymph nodes and sites of chronic inflammation, and of neutrophils into acute inflammatory sites. Acting in cooperation with P-Selectin, L-Selectin mediates the initial interaction of circulating leukocytes with endothelial cells that produces a characteristic 'rolling' of the leukocytes on the endothelium. This initial interaction is followed by a stronger interaction, probably involving E-Selectin, that leads eventually to extravasation through the blood vessel wall into lymphoid tissues and to sites of inflammation (8). Three ligands for L-Selectin have been identified on endothelial cells, all containing O-glycosylated mucin or mucin-like domains (9). The first ligand, GlyCAM-1, is expressed almost exclusively in peripheral and mesenteric lymph node high endothelial venules (9, 10). The second L-Selectin ligand, originally called sgp90, is now known to be CD34 (11). This sialomucin-like glycoprotein shows vascular expression in a wide variety of nonlymphoid tissues, as well as on the capillaries of peripheral lymph nodes (12). The third ligand for L-Selectin is MAdCAM-1, a mucin-like glycoprotein found on mucosal lymph node high endothelial venules (13).

L-Selectin is shed by proteolytic cleavage from the surfaces of lymphocytes and neutrophils *in vitro* following activation by a variety of agents such as phorbol esters, LPS and f-met-leu-phe. *In vivo*, L-Selectin is shed from neutrophils during inflammation (2, 14-16), and it has been suggested that loss of surface L-Selectin might be necessary to allow leukocytes to migrate through the endothelium (14, 15). Soluble L-Selectin (sL-Selectin) derived from lymphocytes is about 62 kDa, while the fragment derived from neutrophils is 75-100 kDa (17). sL-Selectin retains bioactivity, and at high concentrations can inhibit binding of lymphocytes to endothelium (17), suggesting a possible role in modulating this binding *in vivo*. High levels of sL-Selectin have been found in samples drawn from apparently normal individuals (17). A number of studies have reported that levels of sL-Selectin in biological fluids may be elevated or lowered in subjects with a variety of pathological conditions (18-25).

The Human sL-Selectin Immunoassay is a 2 hour solid phase ELISA that is designed to measure sL-Selectin in cell culture supernates, serum, and plasma. It contains recombinant human sL-Selectin and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural human sL-Selectin showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural human sL-Selectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human sL-Selectin has been pre-coated onto a microplate. Standards, samples and Control are pipetted into the wells and any sL-Selectin present is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for human sL-Selectin is then added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of sL-Selectin bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with Sample 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.
- 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 this assay, 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 containers for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Additions at each step of the protocol should be uninterrupted.
- Sodium azide will inactivate the sL-Selectin Conjugate.
- Avoid contact of Substrate with oxidizing agents or metal.
- Substrate should remain colorless until added to the plate. Substrate incubated in the positive wells 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 unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # BBE4B	CATALOG # SBBE4B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
sL-Selectin Microplate	890376	1 plate	6 plates	96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human sL-Selectin.	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.*
sL-Selectin Conjugate	890377	1 vial	6 vials	11 mL/vial of a polyclonal antibody to recombinant human sL-Selectin conjugated to horseradish peroxidase in buffer with red dye and preservatives.	May be stored for up to 1 month at 2-8 °C.*
sL-Selectin Standards	890378-890383	1 vial of each level	6 vials of each level	1.0 mL/vial of recombinant human sL-Selectin with blue dye and preservatives. The concentrations of sL-Selectin are shown on the vial labels.	
sL-Selectin Control	890384	1 vial	6 vials	Lyophilized human serum containing sL-Selectin (*see Precautions*). The concentration of the control should fall within the range specified on the vial label if the assay is valid.	
Sample Diluent	895239	3 vials	18 vials	20 mL/vial of a buffered protein base with blue dye and preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Substrate	895002	1 vial	6 vials	11 mL/vial of stabilized substrate solution (tetramethylbenzidine).	
Stop Solution	895004	1 vial	6 vials	11 mL/vial of 1N hydrochloric acid.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

BBE4B contains sufficient materials to run an ELISA on one 96 well plate.
SBBE4B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PBBE4B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm or 650 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Manifold dispenser or automated microplate washer.
- 500 mL graduated cylinder.

PRECAUTIONS

The sL-Selectin Control contains human serum. This serum was tested at the donor level using FDA licensed methods and was found to be non-reactive for anti-HIV1/2, anti-HCV, HIV-1 antigen, and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, this reagent should be handled as if capable of transmitting infection.

Avoid microbial contamination when removing aliquots from reagent vials.

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.

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.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at 2-8 °C for up to 24 hours or at ≤ -20 °C for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Serum - Allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at 2-8 °C for up to 1 week or at ≤ -20 °C for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at 2-8 °C for up to 1 week or at ≤ -20 °C for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Notes: *Citrate plasma has not been validated for use in this assay.*
Do not use lipemic, grossly hemolyzed, or turbid samples.
Thoroughly mix thawed samples before assay.

SAMPLE PREPARATION

Cell culture supernate samples require at least a 25-fold dilution in Sample Diluent. A suggested 25-fold dilution is 20 µL of sample + 480 µL of Sample Diluent.

Serum and plasma samples must be diluted at least 100-fold in Sample Diluent. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Sample Diluent.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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.

sL-Selectin Control - Reconstitute the sL-Selectin Control immediately before use with 500 µL of distilled or deionized water. Allow the control to sit at room temperature for at least 10 minutes. Mix by gentle swirling or inversion. The sL-Selectin Control must be diluted 100-fold in Sample Diluent prior to assay. A suggested 100-fold dilution is 10 µL Control + 990 µL Sample Diluent.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, Standards, and the sL-Selectin Control be assayed in duplicate.

1. Prepare all reagents, samples, and sL-Selectin Control 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 100 µL Standard, sL-Selectin Control*, or sample* to each well. **Ensure sample addition is uninterrupted and completed within 15 minutes.**
4. Cover the plate with a plate sealer provided and incubate at room temperature for 1 hour.
5. Add 100 µL sL-Selectin Conjugate to each well with sufficient force to ensure mixing.
6. Cover the plate with a new plate sealer and incubate at room temperature for 30 minutes.
7. Aspirate or decant each well and wash, repeating the process five times for a total of six washes. Wash by filling each well with Wash Buffer (400 µL) using a manifold dispenser or autowasher. Complete removal of liquid after each wash 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.
8. Add 100 µL Substrate to each well. Cover the plate with a new plate sealer and incubate at room temperature for 30 minutes.
9. Add 100 µL of Stop Solution to each well. The Stop Solution should be added to the wells in the same order as the Substrate.
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 620 or 650 nm. If wavelength correction is not available, subtract readings at 620 or 650 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 and the sL-Selectin Control require dilution. See Sample Preparation and Reagent Preparation sections.

CALCULATION OF RESULTS

Calculate the mean absorbance values for each set of duplicate Standards.

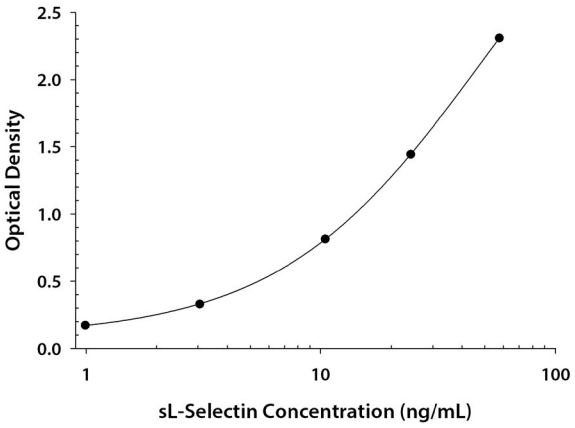
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

For samples and the sL-Selectin Control, the concentration determined from the standard curve must be multiplied by the dilution factor.

It is recommended that the user run the sL-Selectin Control in each assay. If the values obtained are not within the expected range, as stated on the sL-Selectin Control vial label, the assay results may be invalid.

TYPICAL DATA

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



Standard	(ng/mL)	O.D.	Average
0	0.00	0.086 0.087	0.086
1	0.99	0.173 0.171	0.172
2	3.05	0.331 0.327	0.329
3	10.46	0.830 0.800	0.815
4	24.16	1.473 1.413	1.443
5	57.72	2.337 2.281	2.309

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested in replicates of twenty to assess intra-assay precision.

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

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	21	21	21
Mean (ng/mL)	210	881	3025	209	882	3083
Standard deviation	10.5	22.0	124	24.9	77.6	219
CV (%)	5.0	2.5	4.1	11.9	8.8	7.1

RECOVERY

The recovery of sL-Selectin, spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture media (n=2)	100	92-106%
Serum (n=5)	103	88-114%
EDTA plasma (n=5)	102	93-109%

Note: *Heparin plasma samples were found to be equivelant to paired serum samples.*

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of sL-Selectin were diluted in Sample Diluent and assayed in three batches of kits. Samples were diluted prior to assay.

		Cell culture supernates (n=2)	Serum (n=5)	EDTA plasma (n=5)
1:2	Average % of Expected	101	98	98
	Range (%)	101-102	97-101	94-102
1:4	Average % of Expected	———	99	106
	Range (%)	———	97-101	100-111
1:8	Average % of Expected	———	99	107
	Range (%)	———	93-105	93-117

		Cell culture supernates (n=2)
1:25	Average % of Expected	102
	Range (%)	101-104
1:50	Average % of Expected	100
	Range (%)	100-100

Note: *Heparin plasma samples were found to be equivalent to paired serum samples.*

HIGH DOSE HOOK

In common with many sandwich assays, the sL-Selectin assay may show a hook effect with very high levels of analyte; however, levels of sL-Selectin up to 200,000 ng/mL gave O.D. readings greater than the top standard.

SENSITIVITY

The minimum detectable dose of sL-Selectin is typically less than 0.3 ng/mL.

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

CALIBRATION

This assay is standardized against a purified soluble form of recombinant human L-Selectin.

SAMPLE VALUES

Serum/Plasma - Matched serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human sL-Selectin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=35)	663	269-1208
EDTA plasma (n=35)	648	374-1041
Heparin plasma (n=35)	858	500-1451

Note: *Values in EDTA plasma may be slightly decreased compared to paired heparin plasma samples because sL-Selectin is a Ca^{2+}-dependent molecule (26). Results should only be compared within one sample type.*

SPECIFICITY

This assay recognizes natural and recombinant human sL-Selectin. No cross-reactivity was found with recombinant human sP-Selectin or sE-Selectin. Fifty-six additional adhesion molecules were tested and showed no cross-reactivity or interference.

REFERENCES

1. Pigott, R. and C. Power (1993) in *The Adhesion Molecule Facts Book*, Academic Press, p. 100.
2. Tedder, T.F. *et al.* (1990) J. Immunol. **144**:532.
3. Griffin, J.D. *et al.* (1990) J. Immunol. **145**:576.
4. Tedder, T.F. *et al.* (1989) J. Exp. Med. **170**:123.
5. Barclay, A.N. *et al.* (1993) in *The Leukocyte Antigen Facts Book*, Academic Press, p. 348.
6. Bowen, B.R. *et al.* (1989) J. Cell Biol. **109**:421.
7. Lewinsohn, D.M. *et al.* (1987) J. Immunol. **138**:4313.
8. Lasky, L.A. (1993) Current Biol. **3**:680.
9. Shimizu, Y. and S. Shaw (1993) Nature **366**:630.
10. Lasky, L. A. *et al.* (1992) Cell **69**:927.
11. Baumhueter, S. *et al.* (1993) Science **262**:436.
12. Baumhueter, S. *et al.* (1994) Blood **84**:2554.
13. Briskin, M. *et al.* (1993) Nature **363**:461.
14. Jutila, M.A. *et al.* (1989) J. Immunol. **143**:3318.
15. Kishimoto, T.K. *et al.* (1989) Science **245**:1238.
16. Jung, T.M. and M.O. Dailey (1990) J. Immunol. **144**:3130.
17. Schleiffenbaum, B.E. *et al.* (1992) J. Cell Biol. **119**:229.
18. Spertini, O. *et al.* (1992) J. Immunol. Meths. **156**:115.
19. Spertini, O. *et al.* (1994) Blood **84**:1249.
20. Lampeter, E.R. *et al.* (1992) Diabetes **41**:1668.
21. Donnelly, S.C. *et al.* (1994) The Lancet **344**:215.
22. Zetterberg, E. and J. Richter (1994) Eur. J. Haematol. **51**:113.
23. Humbria, A. *et al.* (1994) Arthritis Rheum. **37**:342.
24. Wenisch, C. *et al.* (1995) J.I.D. **171**:1078.
25. Stucki, A. *et al.* (1995) The Lancet **345**:286.
26. Norgard-Sumnicht, K.E. *et al.* (1993) Science **261**:480.