DRG® sL-Selectin (human) (EIA-4875)

CE

As of 1 Aug. 2008 (Vers. 1.0) **USA: RUO**

INTENDED USE

The sL-selectin ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of soluble L-selectin (Leukocyte-Endothelial Cell Adhesion molecule-1) levels in cell culture supernatants, human serum, plasma, amniotic fluid, or other body fluids.

The sL-selectin ELISA is for in vitro use only. Not for therapeutic procedures.

SUMMARY

Leukocyte-Endothelial Cell Adhesion Molecule-1, L-selectin (LECAM-1, MEL-14, LAM-1, LEU-8, TQ1, LEC.CAM-1, DREG.56) belongs to the selectin family of adhesion molecules (4, 10). Together with ELAM-1 (E-selectin) and GMP-140 (P-selectin) L-selectin mediates the initial interactions of leukocytes with endothelial cells (19).

Molecular structure: The extracellular part of all selectins consists of an aminoterminal c-type lectin domain which specifically binds to carbohydrate ligands. This is followed by an EGF-like domain, and in the case of L-selectin, by 2 short consensus repeats similar to the short consensus units in complement regulatory proteins. The transmembrane portion of the molecule is followed by a short cytoplasmic tail.

Selectins guide non-activated polymorphonuclear cells to the areas of inflammation in creating first, loose contacts with the endothelial layer. L-selectin in this aspect mediates rolling of PMN's on endothelial cells (18). The potential binding partners of L-selectin carry a negative charge, probably a sialic acid and/or sulphate, and may contain mannose and fucose (3, 13). In addition, L-selectin may also interact with ELAM-1 which is expressed on cytokine-activated endothelial cells. L-selectin is constitutively expressed on most leukocytes (PMN's, monocytes, lymphocyte subsets) in a seemingly functional form (19, 20). It is required for the binding of lymphocytes to the high endothelial venules of peripheral lymph nodes (and therefore serves as a lymphocyte recirculating receptor) and for the invasion of neutrophils into sites of inflammation (13).

When neutrophils are activated, L-selectin is shed by proteolytic cleveage near the transmembrane span (5). Lymphocytes and monocytes can also shed L-selectin upon activation although the kinetics are significantly lower. A broad range of activating agents including C5a, fMLP, TNF, GM-CSF, IL-8 are effective in inducing this response (6, 15). The shed form of L-selectin (sL-selectin) is functionally active and at high concentrations can inhibit leukocyte attachment to endothelium (16). The main source for sL-selectin in serum seems to be tissue localized leukocytes.

Determination of soluble/circulating L-selectin could provide more detailed insights into the pathological modifications during various diseases (14, 16):

- **allergy:** L-selectin expression is down-modulated on eosinophils recovered from bronchoalveolar lavage fluid after allergen provocation (11).
- **bronchoalveolar lavage (BAL):** BAL transiently promotes PMN/monocyte activation and recruitment to the bronchoalveolar space. The cells respond with a complete shedding of L-selectin when they extravasate from the blood into the bronchoalveolar space (8).
- **deep venous thrombosis (DVT):** A case can be made for the participation of PMN's in the initiation and propagation of venous thrombosis. Probably via L-selectin leukocytes adhere to areas of veins that serve as sites for initiation of thrombi (17).
- **HIV:** patients suffering from HIV-infection showed markedly elevated levels of sL-selectin in serum (16).

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- **insulin-dependent diabetes mellitus (IDDM):** serum levels of L-selectin were found to be elevated in IDDM patients and in subjects at risk for developing IDDM (9).
- **Kawasaki Syndrome:** sL-selectin levels seem to be less than those of normals (16).
- **malignant B-cell populations:** B-cell chronic lymphocytic leukaemia, hairy cell leukaemia and mantle zone lymphoma are L-selectin positive (12).
- **neonatal bacterial infection:** in case of intra-uterine infection lymphocytes obtained from cord blood have a diminished L-selectin expression. This is independent of gestational age, birth weight, umbilical artery pH, hematocrit, leukocyte count, absolute neutrophil count, CRP-level or maternal fever (1).
- **sepsis:** patients suffering from sepsis showed markedly elevated levels of sL-selectin in serum (16). Vascular endothelial injury observed in overwhelming sepsis may be caused by neutrophil-derived enzymes. Adherence to endothelium is a prerequisite for this process. Measurement of sL-selectin may provide further insights into the interrelationship between neutrophil activation and endothelial damage in gram-negative sepsis (7).
- **surgery:** patients undergoing cardiopulmonary bypass surgery may develop an acute post-operative capillary leak, due to endothelial injury inflicted by adherent neutrophils. In those patients L-selectin is completely lost in a small but progressively increasing proportion of PMN's, which could be responsible for the endothelial damage (2).

PRINCIPLES OF THE TEST

An anti-sL-selectin monoclonal coating antibody is adsorbed onto microwells.

sL-selectin present in the sample or standard binds to antibodies adsorbed to the microwells; a HRP-conjugated monoclonal anti-sL-selectin antibody is added and binds to sL-selectin captured by the first antibody.

Following incubation unbound enzyme conjugated anti-sL-selectin is removed during a wash step and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of soluble L-selectin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven sL-selectin standard dilutions and sL-selectin sample concentration determined.

REAGENTS PROVIDED

1	aluminium pouch with **a Microwell Plate coated with Monoclonal Antibody** (murine) to human sL-selectin
1	vial (6 mL) **HRP-Conjugate** anti-sL-selectin monoclonal (murine) antibody, ready to use
2	vials **sL-selectin Standard**, lyophilized, 50ng/ml upon reconstitution; contains preservative
1	bottle (50 mL) **Wash Buffer Concentrate 20x** (PBS with 1% Tween 20); contains preservative
1	bottle (50 mL) **Sample Diluent** (buffered protein matrix).
1	vial (7 mL) **Substrate Solution I** (tetramethyl-benzidine)
1	vial (7 mL) **Substrate Solution II** (0.02 % buffered hydrogen peroxide)
1	vial (12 mL) **Stop Solution** (1M Phosphoric acid)
1	vial (0.4 mL each) **Blue-Dye** and **Green-Dye**
2	adhesive **Plate Covers**

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As of 1 Aug. 2008 (Vers. 1.0) **USA: RUO**

Reagent Labels

STORAGE INSTRUCTIONS

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

SPECIMEN COLLECTION

Cell culture supernatants, human serum, EDTA, or heparinized plasma, amniotic fluid, or other body fluids are suitable for use in the assay. Remove the serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive sL-selectin. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C.

Avoid repeated freeze-thaw cycles. Prior to assay, frozen sera or plasma should be brought to room temperature slowly and mixed gently and properly diluted with Sample Diluent (1:100 see 10.b).

For sample stability refer to 13.5.

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

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PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for in vitro use only and are not for use in therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Reagents containing thimerosal as preservative may be toxic if ingested.
- Avoid contact of substrate solutions with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solutions must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

PREPARATION OF REAGENTS

Except for the sL-selectin Standard (9.2) and the TMB Substrate Solution (9.3) the reagents should be prepared before starting the test procedure.

1.1 Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 mL) of the **Wash Buffer Concentrate** into a clean 1,000 mL graduated cylinder. Bring final volume to 1,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

1.2 Preparation of sL-selectin Standard

Reconstitute **Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete solubility.

1.3 TMB Substrate Solution

Using clean pipettes and containers known to be metal free, dispense an equal volume of **Substrate Solution I** into **Substrate Solution II** and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue colour present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discharged.

The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation.

Substrate preparation by assay size:

Number of Strips	Substrate Solution I (mL)	Substrate Solution II (mL)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

1.4 Addition of colour-giving reagents: Blue-Dye, Green-Dye

In order to help our customers to avoid any mistakes in pipetting the ELISAs, a new tool is offered that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye***) can be added to the reagents according to the following guidelines:

As of 1 Aug. 2008 (Vers. 1.0) **USA: RUO**

1. Diluent: Before sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 mL Diluent	20 µL ***Blue-Dye***
12 mL Diluent	48 µL ***Blue-Dye***
50 mL Diluent	200 µL ***Blue-Dye***
60 mL Diluent	240 µL ***Blue-Dye***

2. HRP-Conjugate: Add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the HRP-conjugate, ready to use.

3 mL HRP-Conjugate	30 µL ***Green-Dye***
6 mL HRP-Conjugate	60 µL ***Green-Dye***

TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

b. Dilute serum or plasma samples 1:100 with Sample Diluent according to one of the following dilution schemes:

   I. 5 µL Sample + 495 µL Sample Diluent alternatively

   II. 10 µL Sample + 90 µL Sample Diluent, take 50 µL of this 1:10 diluted Sample and add 450 µL Sample Diluent.

c. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (murine) to human sL-selectin from holder and store in foil bag with desiccant provided at 2°C - 8°C sealed tightly.

d. Wash the microwell strips twice with approximately 300 µL **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

e. Add 100 µL of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µL of **sL-selectin Standard** (refer to preparation of reagents), in duplicate, into well A1 and A2 (see Figure 1 and 2). Mix the contents by repeated aspiration and ejection and transfer 100 µL to well B1 and B2 respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of sL-selectin Standard dilutions ranging from 25-0.4 ng/mL. Discard 100 µL of the contents from the last microwell used (G1, G2).

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Figure 1: Preparation of sL-selectin standard dilutions:

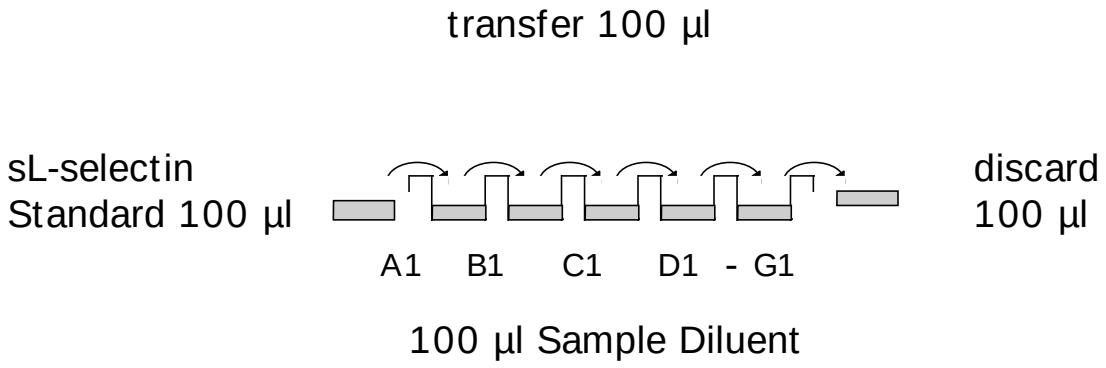

Figure 2: Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (25 ng/mL)	Standard 1 (25 ng/mL)	Sample 1	Sample 1
B	Standard 2 (12.5 ng/mL)	Standard 2 (12.5 ng/mL)	Sample 2	Sample 2
C	Standard 3 (6.3 ng/mL)	Standard 3 (6.3 ng/mL)	Sample 3	Sample 3
D	Standard 4 (3.2 ng/mL)	Standard 4 (3.2 ng/mL)	Sample 4	Sample 4
E	Standard 5 (1.6 ng/mL)	Standard 5 (1.6 ng/mL)	Sample 5	Sample 5
F	Standard 6 (0.8 ng/mL)	Standard 6 (0.8 ng/mL)	Sample 6	Sample 6
G	Standard 7 (0.4 ng/mL)	Standard 7 (0.4 ng/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

f. Add 100 µL of **Sample Diluent** in duplicate to the blank wells.

g. Add 50 µL of **Sample Diluent** to all wells designated for samples.

h. Add 50 µL of each 1:100 diluted **Sample**, in duplicate, to the designated wells and mix the contents.

i. Add 50 µL of **HRP-Conjugate, ready to use,** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature (18°C to 25°C) for 2 hours, if available on a rotator set at 100 rpm.

k. Prepare **TMB Substrate Solution** a few minutes prior to use. (Refer to preparation of reagents).

l. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point d. of the test protocol. Proceed immediately to the next step.

m. Pipette 100 µL of mixed **TMB Substrate Solution** to all wells, including the blank wells.

n. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point o. of this protocol) before positive wells are no longer properly recordable.
It is recommended to add the stop solution when the highest standard has developed a dark blue colour.
Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.

o. Stop the enzyme reaction by quickly pipetting 100 µL of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

p. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the sL-selectin standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

CALCULATION OF RESULTS

Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the sL-selectin concentration on the abscissa. Draw a best fit curve through the points of the graph.

To determine the concentration of circulating sL-selectin for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding sL-selectin concentration.

For samples which have been diluted according to the instructions given in this manual 1:200, the concentration read from the standard curve must be multiplied by the dilution factor (x 200).

It is suggested that each testing facility establishes a control sample of known sL-selectin concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect low sL-selectin levels. Such samples require further dilution e.g. 1:400, 1:800 with Sample Diluent in order to precisely quantitate the actual sL-selectin level.

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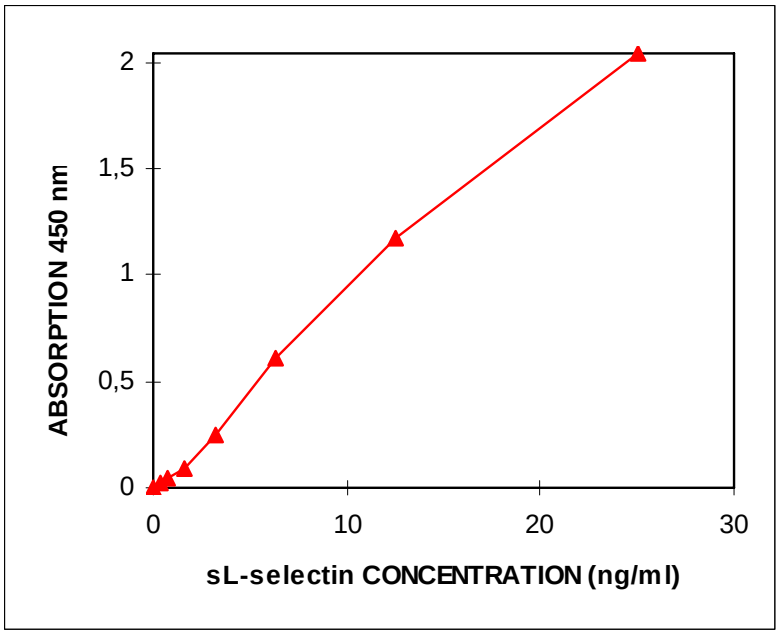
Figure 3: Representative standard curve for sL-selectin ELISA. Recombinant sL-selectin was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the L-selectin ELISA

Measuring wavelength: 450 nm, Reference wavelength: 620 nm

As of 1 Aug. 2008 (Vers. 1.0) **USA: RUO**

Standard	sL-selectin Concentration (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	25	2.008	2.040	2.2
	25	2.071		
2	12.5	1.213	1.167	5.6
	12.5	1.121		
3	6.3	0.608	0.605	0.8
	6.3	0.601		
4	3.2	0.243	0.252	4.8
	3.2	0.260		
5	1.6	0.092	0.095	3.0
	1.6	0.096		
6	0.8	0.042	0.040	9.0
	0.8	0.037		
7	0.4	0.017	0.017	4.3
	0.4	0.016		
Blank	0	0.003	0.003	
	0	0.002		

LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the Sample Diluent.

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PERFORMANCE CHARACTERISTICS

1.5 Sensitivity

The limit of detection of sL-selectin defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.198 ng/mL (mean of 6 independent assays).

1.6 Reproducibility

1.6.1 Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sL-selectin. Two standard curves were run on each plate. Data below show the mean sL-selectin concentration and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 3.7%.

Positive Sample	Experiment	sL-selectin Concentration (ng/ml)	Coefficient of Variation (%)
1	1	1195.3	3.4
	2	1205.7	1.8
	3	1019.7	11.7
2	1	1144.3	1.8
	2	1162.8	2.8
	3	1002.9	2.5
3	1	1411.5	3.3
	2	1407.8	5.8
	3	1245.0	3.6
4	1	722.0	0.5
	2	774.2	2.1
	3	777.8	4.3
5	1	1301.6	1.7
	2	1284.5	6.6
	3	1216.8	6.0
6	1	1021.0	2.1
	2	997.7	2.9
	3	1010.2	6.0
7	1	867.4	2.0
	2	839.2	0.6
	3	808.4	4.3
8	1	603.1	2.3
	2	546.6	2.6
	3	560.0	7.4

1.6.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sL-selectin. Two standard curves were run on each plate. Data below show the mean sL-selectin concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall coefficient of variation was 4.2%.

Sample	sL-selectin Concentration (ng/ml)	Coefficient of Variation (%)
1	1140.2	7.5
2	1103.3	6.5
3	1354.7	5.7
4	758.0	3.4
5	1267.6	2.9
6	1009.7	0.9
7	838.6	2.9
8	569.9	4.2

1.7 Spike Recovery

The spike recovery was evaluated by spiking three levels of recombinant sL-selectin into serum. As shown below recoveries were determined in three independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. Average recovery ranged from 85% to 118% with an overall mean recovery of 99%.

sL-selectin Spike (ng/ml)	Experiment	Recovery % sL-selectin
7	1	85
	2	101
	3	80
3	1	118
	2	109
	3	111
1	1	101
	2	99
	3	91

1.8 Dilution Parallelism

Four serum samples with different levels of sL-selectin were analysed at serial two fold dilutions with 4 replicates each. In the table below the per-cent recovery of expected values is listed. The recovery ranged between 82.2% and 95.6% with an overall recovery of 89.1%.

Sample	Dilution	sL-selectin Concentration (ng/mL) Expected Value	Observed Value	% Recovery of Exp. Value
1	1:200	--	1498.4	--
	1:400	749.2	656.4	87.6
	1:800	374.6	351.2	93.8
	1:1600	187.3	154.0	82.2
2	1:200	--	1362.8	--
	1:400	681.4	613.8	90.1
	1:800	340.7	318.8	93.6
	1:1600	170.3	148.6	87.2
3	1:200	--	1576.0	--
	1:400	788.0	689.6	87.5
	1:800	394.0	374.0	94.9
	1:1600	197.0	166.3	84.4
4	1:200	--	957.9	--
	1:400	478.9	457.6	95.6
	1:800	239.5	202.2	84.4
	1:1600	119.7	104.6	87.4

1.9 Sample Stability

1.9.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked with sL-selectin) were stored at -20°C and thawed several times, and the sL-selectin levels determined. There was no significant loss of sL-selectin concentrations between 0 and 5 freeze-thaw cycles.

1.9.2 Storage Stability

Aliquots of a serum sample (spiked or unspiked with sL-selectin) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the sL-selectin level determined after 24 h.

There was no significant loss of sL-selectin immunoreactivity during storage under above conditions.

1.10 Specificity

The assay recognizes both natural and recombinant human sL-selectin.

To define the specificity of this ELISA several polypeptides were tested for cross reactivity. There was no cross reactivity determined for IL-8, sICAM-1, sTNF-R, TNF-α, TNF-β, CD8, IL-2, IL-2R, IL-6, IL-6R, IL-10, and E-selectin, CD44 and HER-2.

1.11 Expected Values

A panel of 22 sera from healthy blood donors (male and female) was tested for sL-selectin. The detected sL-selectin levels ranged between 487.3 and 1096.3 ng/mL with a mean level of 842 ng/mL and a standard deviation of ± 168.9 ng/mL.

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REAGENT PREPARATION SUMMARY

1. Wash Buffer Add **Wash Buffer Concentrate** 20 x (50 mL) to 950 mL distilled water

2. sL-selectin Standard Reconstitute Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial.

3. TMB Substrate Solution

Number of Strips	Substrate Solution I (mL)	Substrate Solution II (mL)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

TEST PROTOCOL SUMMARY

- Dilute Samples in Sample Diluent
- Wash microwell strips twice with Wash Buffer
- Add 100 µL Sample Diluent, in duplicate, to standard wells
- Pipette 100 µL solubilized sL-selectin Standard in duplicate into the first wells and create standard dilutions ranging from 25 to 0.4 ng/mL by transferring 100 µL from well to well. Discard 100 µL from the last well.
- Add 100 µL Sample Diluent to the blank wells
- Add 50 µL Sample Diluent to the sample wells
- Add 50 µL diluted Sample in duplicate to designated wells

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- Add 50 µL of HRP-Conjugate, ready to use, to all wells
- Cover microwell strips and incubate 2 hours at room temperature (18°C to 25°C)
- Prepare TMB Substrate Solution few minutes prior to use
- Empty and wash microwell strips 3 times with Wash Buffer
- Add 100 µL of mixed TMB Substrate Solution to all wells including blank wells
- Incubate the microwell strips for about 15 minutes at room temperature (18°to 25°C).
- Add 100 µL Stop Solution to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: For samples which have been diluted according to the instructions given in this manual 1:200, the concentration read from the standard curve must be multiplied by the dilution factor (x200). Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sL-selectin levels. Such samples require further dilution of 1:400 - 1:800 with Sample Diluent in order to precisely quantitate the actual sL-selectin level.

SYMBOLS USED WITH DRG ASSAY´S

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
Symbol	**Portugues**	**Dansk**	**Svenska**	**Ελληνικά**	

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	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
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