Human sL-selectin ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human sL-selectin

Catalog Numbers BMS206 and BMS206TEN

Pub. No. MAN0016513 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Human sL-selectin ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human L-selectin.

Summary

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

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. sL-selectin in this aspect mediates rolling of PMN's on endothelial cells. The potential binding partners of sL-selectin carry a negative charge, probably a sialic acid and/or sulphate, and may contain mannose and fucose. In addition, sL-selectin may also interact with ELAM-1, which is expressed on cytokine-activated endothelial cells. sL-selectin is constitutively expressed on most leukocytes (PMN's, monocytes, lymphocyte subsets) in a seemingly functional form. 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. When neutrophils are activated, sL-selectin is shed by proteolytic cleveage near the transmembrane span. Lymphocytes and monocytes can also shed sL-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. The shed form of sL-selectin (L-selectin) is functionally active and at high concentrations can inhibit leukocyte attachment to endothelium. The main source for sL-selectin in serum seems to be tissue localized leukocytes.

For literature update refer to our website.

Principles of the test

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

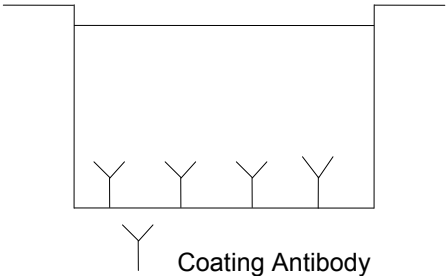

Fig. 1 Coated microwell.

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

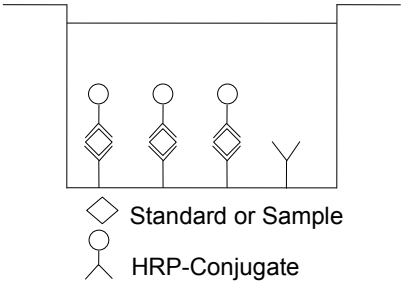

Fig. 2 First incubation.

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

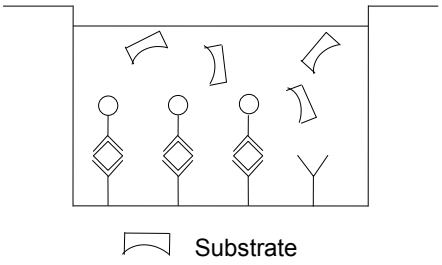

Fig. 3 Second incubation.

A colored product is formed in proportion to the amount of human sL-selectin present in the sample or standard. The reaction is terminated by addition of acid, and absorbance is measured at 450 nm. A standard curve is prepared from 7 human sL-selectin standard dilutions and human sL-selectin concentration determined.

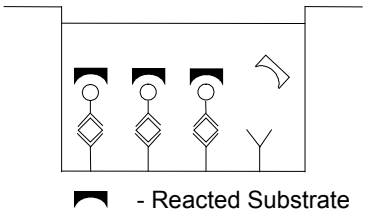

Fig. 4 Stop reaction.

Reagents provided

Reagents for human sL-selectin ELISA BMS206 (96 tests)

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human L-selectin

1 vial (6 mL) HRP-Conjugate anti-human sL-selectin monoclonal antibody, ready to use

2 vials human sL-selectin Standard lyophilized, 50 ng/mL upon reconstitution

1 bottle (50 mL) Sample Diluent

1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

2 Adhesive Films

Reagents for human sL-selectin ELISA BMS206TEN (10x96 tests)

10 aluminum pouches with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human L-selectin

10 vials (6 mL) HRP-Conjugate anti-human sL-selectin monoclonal antibody

10 vials human sL-selectin Standard lyophilized, 50 ng/mL upon reconstitution

10 bottles (50 mL) Sample Diluent

3 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

10 Adhesive Films

Storage instructions – ELISA kit

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.

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, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Cell culture supernatant, serum, and plasma (EDTA, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible *Hook Effect* due to high sample concentrations (see "Calculation of results" on page 4).

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

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human L-selectin. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (refer to "Sample stability" on page 5).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and 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 regression analysis

Precautions for use

- All reagents 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 statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or 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 pipet 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 samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that 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 acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples 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

1. Buffer Concentrate should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrate, warm it gently until they have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2°C to 25°C. The Wash Buffer (1x) is stable for 30 days.
4. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

Human sL-selectin standard

1. Reconstitute human sL-selectin standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 50 ng/mL).
2. Allow the standard to reconstitute for 10–30 minutes. Mix well prior to making dilutions.

The standard has to be used immediately after reconstitution and cannot be stored.

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of reconstituted (concentration of standard = 50 ng/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 25 ng/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 5).

   Sample Diluent serves as blank.

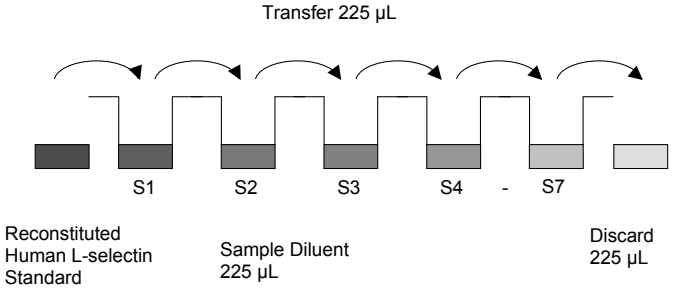

Fig. 5 Dilute standards - tubes.

Test protocol

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

1. Predilute your samples before starting with the test procedure. Dilute serum, plasma, and cell culture samples 1:100 with Sample Diluent according to the following scheme:
   - Dilution 1: 10 µL sample + 90 µL Sample Diluent
   - Dilution 2: 50 µL of dilution 1 + 450 µL Sample Diluent
2. 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 from holder and store in foil bag with the desiccant provided at 2°C to 8°C sealed tightly.
3. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
4. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see "External standard dilution" on page 3):

   Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of prepared standard (see "Human sL-selectin standard" on page 3, concentration = 50.0 ng/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 25.0 ng/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 6). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human sL-selectin standard dilutions ranging from 50.0 to 0.4 ng/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

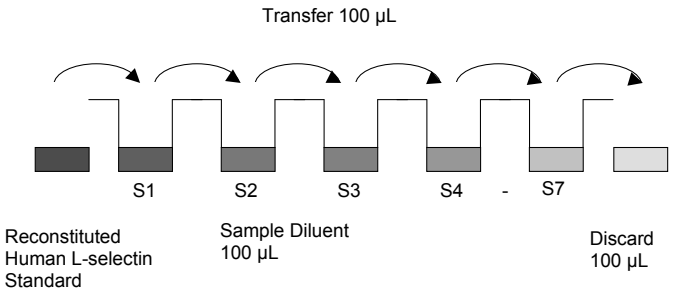

Fig. 6 Dilute standards - microwell plate.

In case of an external standard dilution (see "External standard dilution" on page 3), pipette 100 µL of these standard dilutions (S1–S7) in the standard wells according to Table 1.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 25.0 ng/mL	Standard 1 25.0 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

5. Add 100 µL of Sample Diluent in duplicate to the blank wells.
6. Add 50 µL of Sample Diluent to the sample wells.
7. Add 50 µL of each sample in duplicate to the sample wells.
8. Add 50 µL of HRP-Conjugate to all wells.

9. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 2 hours on a microplate shaker.
10. Remove adhesive film and empty wells. Wash microwell strips 3 times according to step 3 of the test protocol. Proceed immediately to the next step.
11. Pipette 100 µL of TMB Substrate Solution to all wells.
12. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next step of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.
13. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. 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°C to 8°C in the dark.
14. 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 standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human sL-selectin concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human 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 human sL-selectin concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:200 (1:100 external predilution, 1:2 dilution on the plate: 50 µl sample + 50 µl Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 200).
- Calculation of 1:100 prediluted samples with a concentration exceeding standard 1 may result in incorrect, low human L-selectin levels (Hook Effect). Such samples require further external predilution according to expected human sL-selectin values with Sample Diluent in order to precisely quantitate the actual human sL-selectin level.
- It is suggested that each testing facility establishes a control sample of known human sL-selectin concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 7.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

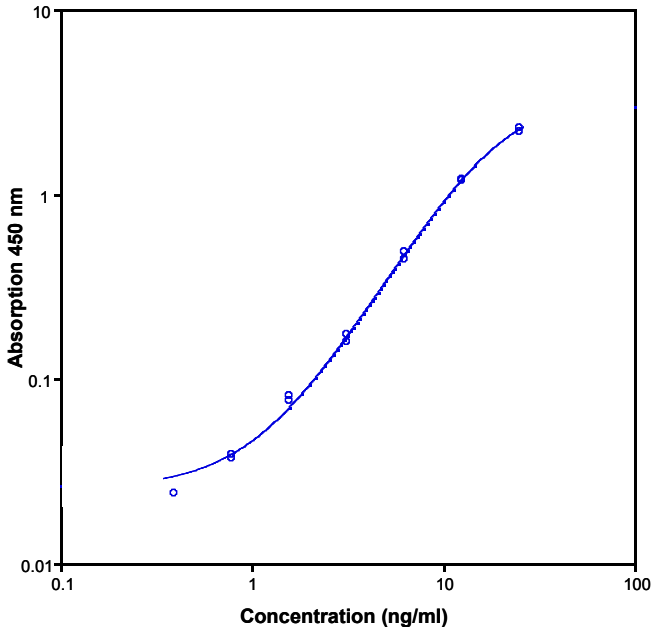

Fig. 7 Representative standard curve for Human sL-selectin ELISA Kit. Human L- selectin was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the Human sL-selectin ELISA Kit.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human L-selectin concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	25.0	2.273 2.183	2.228	2.0
2	12.5	1.207 1.172	1.189	1.5
3	6.3	0.489 0.440	0.465	5.4
4	3.2	0.174 0.158	0.166	4.9
5	1.6	0.081 0.076	0.078	2.9
6	0.8	0.039 0.037	0.038	2.0
7	0.4	0.024 0.024	0.024	0
Blank	0.0	0.015 0.014	0.015	3.4

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Because 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. Empty wells completely before dispensing fresh wash solution, 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 antibodies (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 analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Performance characteristics

Sensitivity

The limit of detection of human sL-selectin defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.198 ng/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human L-selectin. Two standard curves were run on each plate. Data below show the mean human sL-selectin concentration and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 3.7%.

Table 3 The mean human sL-selectin concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean concentration (ng/mL)	Coefficient of variation (%)
1	1	1195.3	3.4
	2	1205.7	1.8
	3	1,019.7	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

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human L-selectin. Two standard curves were run on each plate. Data below show the mean human sL-selectin concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall inter-assay coefficient of variation was 4.2%.

Table 4 The mean human sL-selectin concentration and the coefficient of variation of each sample.

Sample	Mean 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

Spike recovery

The spike recovery was evaluated by spiking 3 levels of human L-selectin into serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The amount of endogenous human sL-selectin in unspiked serum was subtracted from the spike values. The recovery ranged from 85–118% with an overall mean recovery of 99%.

Experiment	Spike high (%)	Spike medium (%)	Spike low (%)
1	85	118	101
2	101	109	99
3	80	111	91

Dilution parallelism

Serum samples with different levels of human sL-selectin were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged from 82% to 96% with an overall recovery of 89%.

Sample	Dilution	Expected human sL-selectin concentration (ng/mL)	Observed human sL-selectin concentration (ng/mL)	Recovery of expected human sL-selectin concentration (%)
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

Sample stability

Freeze-thaw stability

Aliquots of serum samples (spiked or unspiked) were stored at –20°C and thawed 5 times, and the human sL-selectin levels determined. There was no significant loss of human sL-selectin immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum samples (spiked or unspiked) were stored at –20°C, 2°C to 8°C, room temperature, and at 37°C, and the human sL-selectin level determined after 24 hours. There was no significant loss of human L-selectin immunoreactivity detected during storage under above conditions.

Specificity

The assay detects both natural and recombinant human L-selectin. The interference of IL-8, ICAM-1, TNF-R, TNFα, TNFβ, CD8, IL- 2, IL-2R, IL-6, IL-6R, IL-10, E-selectin, CD44, and HER-2 was evaluated by spiking these proteins at physiologically relevant concentrations into a human sL-selectin positive serum. No cross-reactivity was detected.

Expected values

A panel of 22 sera samples from randomly selected apparently healthy donors (males and females) was tested for human L-selectin. The detected human sL-selectin levels ranged between 487.3 ng/mL and 1096.3 ng/mL with a mean level of 842.0 ng/mL and a standard deviation of 168.9 ng/mL.

Reagent preparation summary

Wash buffer (1x)

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

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

Human sL-selectin standard

Reconstitute lyophilized human sL-selectin standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

Note: If instructions in this protocol have been followed, samples have been diluted 1:200 (50 µL 1:100 prediluted sample + 50 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 200).

1. Predilute sample with Sample Diluent 1:100.
2. Determine the number of microwell strips required.
3. Wash microwell strips twice with Wash Buffer.
4. Standard dilution on the microwell plate: Add 100 µL Sample Diluent, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

   Alternatively, external standard dilution in tubes (see "External standard dilution" on page 3): Pipette 100 µL of these standard dilutions in the microwell strips.
5. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
6. Add 50 µL Sample Diluent to sample wells.
7. Add 50 µL sample in duplicate, to designated sample wells.
8. Add 50 µL HRP-Conjugate, ready to use to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18°C to 25°C).
10. Empty and wash microwell strips 3 times with Wash Buffer.
11. Add 100 µL of TMB Substrate Solution to all wells.
12. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
13. Add 100 µL Stop Solution to all wells.
14. Blank microwell reader and measure color intensity at 450 nm.

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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