PRODUCT INFORMATION & MANUAL

Human sL-selectin FlowCytomix Simplex Kit

BMS80206FF

For research use only.
Not for diagnostic or therapeutic procedures.

eBioscience®
An Affymetrix Company

Human sL-selectin FlowCytomix Simplex Kit

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This human sL-selectin Simplex Kit must be used in combination with FlowCytomix human Basic Kit BMS8420FF. For test procedure, measurement and calculation of results please refer to FlowCytomix human Basic Kit BMS8420FF manual.

1 REAGENTS PROVIDED

1 vial (175 µl) **Fluorescent Beads** (20x) coated with monoclonal antibody to human sL-selectin, Bead Population **A3**

2 vials human sL-selectin **Standard** (lyophilized): 2 µg/ml upon reconstitution

1 vial (350 µl) **Biotin-Conjugate** (20x) anti-human sL-selectin monoclonal antibody

2 INTENDED USE

BMS80206FF is a bead based Analyte Detection System for quantitative detection of human sL-selectin by Flow Cytometry. **BMS80206FF is for research use only. Not for use in diagnostic or therapeutic procedures.**

Please note: Samples must be **prediluted 1:50** in Assay Buffer (included in the Basic Kit BMS8420FF) before starting the test procedure.
In combination with other Simplex Kits it is recommended evaluating both, an undiluted and a 1:50 prediluted sample.

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 molecule. Together with ELAM-1 (E-selectin) and GMP-140 (P-selectin) L-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. L-selectin in this aspect mediates rolling of PMN's on endothelial cells. The potential binding partners of L-selectin carry a negative charge, probably a sialic acid and/or sulphate, and may contain mannose and fucose. 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. 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, L-selectin is shed by proteolytic cleveage near the transmembrane span. 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. The shed form of L-selectin (sL-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.

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

- **allergy:** L-selectin expression is down-modulated on eosinophils recovered from bronchoalveolar lavage fluid after allergen provocation.

- **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.

- **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.

- **HIV:** patients suffering from HIV-infection showed markedly elevated levels of sL-selectin in serum.

- **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.

- **Kawasaki Syndrome:** sL-selectin levels seem to be less than those of normals.

- **malignant B-cell populations:** B-cell chronic lymphocytic leukaemia, hairy cell leukaemia and mantle zone lymphoma are L-selectin positive.

- **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.
- **sepsis:** patients suffering from sepsis showed markedly elevated levels of sL-selectin in serum. 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.

- **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.

For literature update refer to **www.eBioscience.com**

3 STORAGE INSTRUCTIONS – SIMPLEX KIT

Store kit and components at 2 to 8°C. 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.

4 SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma (EDTA, citrate, 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 chapter 6.4).

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

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human sL-selectin. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

5 REPRESENTATIVE STANDARD CURVE

Table 1

Representative standard curve.

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

Concentration (pg/ml)	Fluorescent Intensity (FI)
100000	364.88
33333	195.33
11111	65.81
3704	14.65
1235	2.70
412	0.51
137	0.17
0	0.11

6 PERFORMANCE CHARACTERISTICS

Assay performance data presented in this manual was generated in house, and is considered typical for a routine experiment in our laboratories. Each laboratory using this product should establish its own performance characteristics, and these may vary from those presented in the manual.

6.1 Sensitivity

The limit of detection of human sL-selectin defined as the concentration resulting in a fluorescent intensity significantly higher than that of the dilution medium (mean + 2 standard deviations) was determined to be 70 pg/ml.

The value shown depends on the type of flow cytometer used for analysis as well as on the respective instrument setup. The value shown is for guidance only. Optimum results for each machine can be achieved by following the instrument set up process.

6.2 Reproducibility

6.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of human sL-selectin (high, medium and low concentration, medium low and low concentration). 2 standard curves were run on each plate. Data below show the mean intra-assay coefficient of variation for human sL-selectin (see Table 2). It has been calculated to be 2.6%.
Individual user data may vary due to differences in protein content of serum/plasma pools or individual donor serum/plasma.

Table 2
The coefficient of variation of the human sL-selectin concentration calculated for each sample.

	CV Sample 1 high (%)	**CV Sample 2 medium (%)**	**CV Sample 3 low (%)**	**Mean intra-assay CV (%)**
sL-selectin	2.2	2.7	2.9	**2.6**

6.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of human sL-selectin (high, medium high, medium low and low concentration). 2 standard curves were run on each plate. Data below (see Table 3) show the mean inter-assay coefficient of variation for human sL-selectin, calculated on 12 determinations of each sample. It has been calculated to be 1.2%.
Individual user data may vary due to differences in protein content of serum/plasma pools or individual donor serum/plasma.

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

	CV Sample 1 high (%)	**CV Sample 2 medium high (%)**	**CV Sample 3 medium low (%)**	**CV Sample 4 low (%)**	**Mean inter-assay CV (%)**
sL-selectin	1.3	0.6	1.2	1.7	**1.2**

6.3 Specificity

Cross reactivity was tested with combinable analytes of Simplex and Multiplex Assays. There was no detectable cross reactivity observed. (For detailed information refer to “Combination Table“ on www.eBioscience.com.)

6.4 Hook Effect

1:50 prediluted samples Samples with expected concentrations two fold higher than the concentration of highest standard should be diluted 10 fold in Assay Buffer (1x) before assay performance to prevent false negative results due to a possible “Hook Effect”.

7 ORDERING INFORMATION

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