

PRODUCT INFORMATION AND MANUAL

***FlowCytomix
Human sP-selectin Simplex Kit***

BMS8219/2FF

For research use only.

Not for diagnostic or therapeutic procedures.

96 Tests

**Human sP-selectin
BMS8219/2FF Simplex Kit**



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This human sP-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 sP-selectin, Bead Population **B4**
- 2 vials human sP-selectin **Standard** (lyophilized): 40 µg/ml upon reconstitution
- 1 vial (350 µl) **Biotin-Conjugate** (20x) anti-human sP-selectin monoclonal antibody

2 INTENDED USE

BMS8219/2FF is a bead based Analyte Detection System for quantitative detection of human sP-selectin by Flow Cytometry.

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3 SUMMARY

P-selectin (CD62, GMP-140, PADGEM) belongs to the selectin family of adhesion molecules. P-selectin acts as a receptor that supports binding of leukocytes to activated platelets and endothelium. P-selectin-mediated adhesive interactions operate in conjunction with cell-cell interactions directed by related molecules and are likely to be important in both hemostatic and inflammatory processes.

P-selectin is located in membranes of granules in unstimulated platelets and redistributed to the cell surface upon platelet activation. P-selectin is also present in endothelial cells in membranes of Weibel-Palade bodies and megakaryocytes. Surface appearance of P-selectin is very rapid, but transient declining to basal level within short time following stimulation.

P-selectin is a 140 kDa protein that is highly glycosylated. The cDNA-derived amino acid sequence predicts a molecule with a series of cysteine-rich domains. Like the other selectins P-selectin contains an N-terminal Ca^{2+} dependent lectin-like domain and an EGF-like motif which is followed by nine consensus repeats, a transmembrane domain, and a short cytoplasmic tail.

The human gene for P-selectin is located on chromosome 1q21-24. P-selectin is a receptor for neutrophils and monocytes, recognizing oligosaccharide structures on the target cells.

The physiologic role of P-selectin might be the mediation of initial leukocyte adhesion to activated endothelium during acute inflammation. It may work in concert with E-selectin to direct early, regionally specific adherence of neutrophils and monocytes at sites of acute inflammation. A soluble form of P-selectin found in serum and plasma has been described which might represent a proteolytic fragment or more likely a soluble splice variant lacking the transmembrane domain.

Soluble P-selectin is a potentially important molecule to provide more detailed insight into pathological situations. Excessive accumulation of neutrophils on the endothelial surface accompanied by high exposure of P-selectin has been implicated in a number of inflammatory disorders, including adult respiratory distress syndrome, acute lung injury, ischemia-reperfusion injury, Gram-negative septic shock, thrombotic diseases and rheumatoid arthritis.

Malignant cells were shown to express receptors for P-selectin suggesting an important role for P-selectin in tumor formation and metastasis. Platelets have also been shown to promote tumor metastasis.

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

4 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.

5 SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma (EDTA, citrate) 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 7.4).

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 sP-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.

6 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 (ng/ml)	Fluorescent Intensity (FI)
2000	602.6
667	444.1
222	231.1
74	61.8
25	10.5
8	2.7
3	1.9
0	1.5

7 PERFORMANCE CHARACTERISTICS

Assay performance data presented in this manual was generated by Bender MedSystems, 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.

7.1 Sensitivity

The limit of detection of human sP-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 1.2 ng/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.

7.2 Reproducibility

7.2.1 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 sP-selectin (high, medium high, 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 sP-selectin (see Table 2). It has been calculated to be 7.9%.

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 sP-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 intra- assay CV (%)
h sP-selectin	6.9	9.4	9.7	5.5	7.9

7.2.2 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 sP-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 sP-selectin, calculated on 12 determinations of each sample. It has been calculated to be 4.3%.

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 sP-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 (%)
h sP-selectin	5.9	4.6	4.3	2.2	4.3

7.3 Specificity

Cross reactivity was tested with combinable analytes of Simplex and Multiplex Assays from Bender MedSystems. There was no detectable cross reactivity observed.

(For detailed information refer to "Combination Table" on www.bendermedsystems.com.)

7.4 Hook Effect

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".

8 ORDERING INFORMATION

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