

*PRODUCT INFORMATION AND MANUAL*

***FlowCytomix  
Human sVCAM-1 Simplex Kit***

***BMS8232/2FF***

For research use only.

Not for diagnostic or therapeutic procedures.

96 Tests

**Human sVCAM-1  
BMS8232/2FF Simplex Kit**



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**This human sVCAM-1 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 sVCAM-1, Bead Population **B5**
- 2 vials human sVCAM-1 **Standard** (lyophilized): 40 µg/ml upon reconstitution
- 1 vial (350 µl) **Biotin-Conjugate** (20x) anti-human sVCAM-1 monoclonal antibody

## **2 INTENDED USE**

BMS8232/2FF is a bead based Analyte Detection System for quantitative detection of human sVCAM-1 by Flow Cytometry. **BMS8232/2FF is for research use only. Not for use in diagnostic or therapeutic procedures.**

**Please note:** Samples must be **prediluted 1:20** 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 1:20 prediluted sample.

### 3 SUMMARY

The vascular cell adhesion molecule-1 (VCAM-1) or CD106 is a member of the immunoglobulin gene superfamily. The initial molecular cloning of VCAM-1 reported six extracellular Ig-like domains (6D VCAM-1).

This 6D VCAM-1 arises due to alternative splicing from a seven-domain VCAM-1 (7D VCAM-1). 7D VCAM-1 is the dominant form expressed by cultured human endothelial cells. Domains 1 through 3 are highly homologous to domains 4 through 6, suggesting that they arose by gene duplication. The cDNA of 7D VCAM-1 predicts a core protein of approximately 81 kD with seven potential N-linked glycosylation sites. Upon complete glycosylation the mature protein has a molecular weight of approximately 102 kD. This observation is in general agreement with immunoprecipitation studies that show a protein of approximately 110 kD on cytokine-activated endothelium.

Murine and rat VCAM-1 have been cloned. In contrast to ICAM-1, VCAM-1 appears to have been highly conserved through evolution. Both rat and mouse VCAM-1 are highly homologous at the protein level to the human VCAM-1 (77% and 76%, respectively). VCAM-1 supports the adhesion of lymphocytes, monocytes, natural killer cells, eosinophils, and basophils through its interaction with leukocyte very late antigen-4 (VLA-4). VCAM-1/VLA-4 interaction mediates firm adherence of circulating non-neutrophilic leukocytes to endothelium. VCAM-1 also participates in leukocyte adhesion outside of the vasculature, mediating precursor lymphocyte adhesion to bone marrow stromal cells and B cell binding to lymph node follicular dendritic cells.

VCAM-1 is not constitutively expressed on endothelium, but can be up-regulated in vitro in response to LPS, TNF- $\alpha$ , and IL-1, as well as to interferon- $\gamma$  and IL-4.

VCAM-1 is also present on tissue macrophages, dendritic cells, bone marrow fibroblasts, myoblasts and myotubes.

A soluble form of VCAM-1 (sVCAM-1) has been described. Soluble VCAM-1 levels have been found in the serum of healthy individuals and increased levels of sVCAM-1 can be detected in several diseases:

Cancer: Ovarian, gastric-intestinal, renal, bladder cancer, non-Hodgkin's lymphoma, breast cancer (cyst fluid);

Autoimmune diseases: Multiple sclerosis (cerebrospinal fluid), systemic sclerosis, systemic lupus erythematosus, rheumatoid arthritis.

Infections: Sepsis, meningitis, malaria;

Inflammation: Vasculitis, alcoholic cirrhosis, primary biliary cirrhosis, Wegener's granulomatosis;

Others: Impaired renal function, haemodialysis, hyperthyroidism, renal allograft.

For literature update refer to **[www.bendermedsystems.com](http://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, 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 sVCAM-1. 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.*

<b>Concentration (ng/ml)</b>	<b>Fluorescent Intensity (FI)</b>
2000	507.7
667	367.3
222	204.7
74	74.6
25	13.7
8	2.9
3	0.9
0	0.5

## 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 sVCAM-1 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 0.9 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 sVCAM-1 (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 sVCAM-1 (see Table 2). It has been calculated to be 4.9%.

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 sVCAM-1 concentration calculated for each sample.

	<b>CV Sample 1 high (%)</b>	<b>CV Sample 2 medium high (%)</b>	<b>CV Sample 3 medium low (%)</b>	<b>CV Sample 4 low (%)</b>	<b>Mean intra- assay CV (%)</b>
<b>h sVCAM-1</b>	7.8	3.1	3.0	5.7	<b>4.9</b>

### 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 sVCAM-1 (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 sVCAM-1, calculated on 12 determinations of each sample. It has been calculated to be 6.6%.

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 sVCAM-1 concentration calculated for each sample.

	<b>CV Sample 1 high (%)</b>	<b>CV Sample 2 medium high (%)</b>	<b>CV Sample 3 medium low (%)</b>	<b>CV Sample 4 low (%)</b>	<b>Mean inter- assay CV (%)</b>
<b>h sVCAM-1</b>	12.8	5.6	4.5	3.6	<b>6.6</b>

### 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](http://www.bendermedsystems.com).)

### 7.4 Hook Effect

1:20 prediluted 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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