PRODUCT INFORMATION & MANUAL

Human sICAM-1 FlowCytomix Simplex Kit

BMS80201FF

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

eBioscience®
An Affymetrix Company

Human sICAM-1 FlowCytomix Simplex Kit

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This human sICAM-1 Simplex Kit PE must be used in combination with FlowCytomix human Basic Kit PE BMS8421FF. For test procedure, measurement and calculation of results please refer to FlowCytomix human Basic Kit PE BMS8421FF manual.
In case this Simplex Kit PE is combined with other Simplex Kits (not PE Simplex Kits) human Basic Kit for Simplex Kits BMS8420FF is required.

1 REAGENTS PROVIDED

1 vial (175 µl) **Fluorescent Beads** (20x) coated with monoclonal antibody to human sICAM-1, Bead Population **A11**

2 vials human sICAM-1 **Standard** (lyophilized): 80 µg/ml upon reconstitution

1 vial (350 µl) **PE-Conjugate** (20x) anti-human sICAM-1 monoclonal antibody

2 INTENDED USE

BMS80201FF is a bead based Analyte Detection System for quantitative detection of human sICAM-1 by Flow Cytometry. **BMS80201FF is for research use only. Not for use in diagnostic or therapeutic procedures.**

3 SUMMARY

Intercellular Adhesion Molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family and functions as a ligand for the Lymphocyte Function-Associated Antigen-1 (LFA-1), an alpha-beta-complex that is a member of the leukocyte integrin family of cell-cell and cell-matrix receptors. This family consists of the leukocyte adhesion glycoproteins LFA-1 which mediates lymphocyte adhesion, Mac-1 which mediates granulocyte adhesion and p150,95.

ICAM-1 is a single-chain glycoprotein with a polypeptide core of 55 kD that can be expressed on non-hematopoietic cells of many lineages such as vascular endothelial cells, thymic epithelial cells, other epithelial cells and fibroblasts and on hematopoietic cells such as tissue macrophages, mitogen-stimulated T-lymphoblasts, germinal center B-cells and dendritic cells in tonsils, lymph nodes and Peyer's patches. ICAM-1 is inducible on fibroblasts and endothelial cells by inflammatory mediators such as IL-1, TNF and IFN-gamma within few hours and is correlated to the infiltration of lymphocytes into inflammatory lesions. ICAM-1 seems to be the initial marker of inflammatory reactions and is expressed prior to, and to a greater extent than is HLA-DR.

The role of ICAM-1 as a disease marker has been demonstrated for a number of different indications and pathological situations.

ICAM-1 upregulation in allergic airway inflammation is responsible for the recruitment of activated leukocytes and the pathogenesis of allergic rhinitis.

In allergic contact dermatitis ICAM-1 on keratinocytes was induced already 4 hours after application of the allergic patch test.

In bladder cancer there is a direct correlation between constitutive ICAM-1 expression and the histopathologic grade of the tumor. Sera of GI-cancer patients with liver metastasis showed significant higher sICAM-1 levels than those of patients without metastasis.

ICAM-1 is expressed on malignant cells in myeloid as well as B lymphoid malignancies. In lymphoproliferative disorders ICAM-1 is related to the degree of malignancy. In HTLV-1 associated myelopathy, and adult T-cell leukemia sICAM-1 serum levels are elevated.

Patients with malignant melanoma have significantly increased serum levels of sICAM-1, which is of prognostic importance.

Significantly elevated concentrations of sICAM-1 are detected in HIV-1 infected persons.
In malaria tropica ICAM-1 serves in the adhesion of infected erythrocytes to the capillary endothelium which event is important in the pathogenesis of cerebral malaria.
sICAM-1 is a good prognostic parameter of responsiveness of hepatitis B infection to IFNγ-therapy.

ICAM-1 seems to provide the mechanism crucial to allograft rejection of the cornea.
Expression of ICAM-1 is also increased during rejection on the capillary endothelium, the myocardial membrane, and the endocardium of the transplanted heart.
sICAM-1 serum levels significantly increased with acute renal graft rejection. Measuring sICAM-1 is helpful in discriminating rejection from Cyclosporine-A intoxication of the transplanted kidney.
Strong expression of ICAM-1 is also seen in patients with acute rejection versus stable liver transplants, or patients with non-rejection complications.

Serum levels of circulating ICAM-1 and L-selectin were found elevated in insulin-dependent diabetes mellitus (IDDM) and in subjects at risk of IDDM.

Significant elevation of serum ICAM-1 has been demonstrated in anterior uveitis in intermediate uveitis and in patients with sarcoidosis.

In the first 12-24 hours of monitoring acute myocardial infarction a decrease of sICAM-1 is measurable. This can provide prognostic significance to sICAM-1 also for myocardial ischemia and reperfusion.

Increased glomerular ICAM-1 expression is seen in early cases of different forms of glomerulonephritis and tubular de novo expression of ICAM-1 shows a strong correlation with disease activity.

In asthma ICAM-1 is upregulated on inflamed airway epithelium and bronchial endothelium, thereby mediating eosinophil adhesion. sICAM-1

is significantly elevated in the sera of patients with idiopathic pulmonary fibrosis or sarcoidosis.

sICAM-1 is a reliable marker for an inflammatory process within the central nervous system which is associated with blood: CSF barrier disturbance.

Soluble ICAM-1 is not detectable in most midtrimester amniotic fluid samples but when present is significantly related to intrauterine growth retardation and elevated midtrimester levels of maternal serum alpha fetoprotein.

Elevated levels of sICAM-1 correlate with the activity of the rheumatoid arthritis.

In psoriasis ICAM-1 on keratinocytes shows strong correlation with severity of disease and decreases under successful therapy. Before treatment sICAM-1 levels are significantly elevated compared to healthy controls.

For literature update refer to **www.eBioscience.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 (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 sICAM-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.

Concentration (ng/ml)	Fluorescent Intensity (FI)
4000	210.3
1333	115.9
444	40.5
148	11.6
49	5.3
16	3.8
5	3.5
0	3.3

7 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.

7.1 Sensitivity

The limit of detection of human sICAM-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 5.3 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 sICAM-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 sICAM-1 (see Table 2). It has been calculated to be 2.9%.
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 sICAM-1 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 sICAM-1	2.9	4.1	2.5	2.0	**2.9**

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 sICAM-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 sICAM-1, calculated on 12 determinations of each sample. It has been calculated to be 2.0%.
Individual user data may vary due to differences in protein content of human sICAM-1 serum/plasma pools or individual donor serum/plasma.

Table 3
The coefficient of variation of the human sICAM-1 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 sICAM-1	4.2	1.1	1.6	1.0	**2.0**

7.3 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.)

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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