PRODUCT INFORMATION & MANUAL

Human sAPO-1/Fas FlowCytomix Simplex Kit

BMS80245FF

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

eBioscience®
An Affymetrix Company

Human sAPO-1/Fas FlowCytomix Simplex Kit

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This human sAPO-1/Fas 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 sAPO-1/Fas, Bead Population **A3**

2 vials human sAPO-1/Fas **Standard** (lyophilized): 500 ng/ml upon reconstitution

1 vial (350 µl) **Biotin-Conjugate** (20x) anti-human sAPO-1/Fas monoclonal antibody

2 INTENDED USE

BMS80245FF is a bead based Analyte Detection System for quantitative detection of human sAPO-1/Fas by Flow Cytometry. **BMS80245FF is for research use only. Not for use in diagnostic or therapeutic procedures.**

Please note: Samples must be **prediluted 1:5** 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 1:5 prediluted sample.

3 SUMMARY

Programmed cell death or Apoptosis is the most common form of eukaryotic cell death and is found during tumor regression and embryonic development. In the process of selection and eliminiation of autoreactive B and T cells Apoptosis is an important process and therefore a prerequisite for the homeostasis of the immune system. Apoptosis is characterized by changes in cellular morphology (e.g. nuclear condensation, membrane bleeding) and biochemically by rapid induction on DNA fragmentation.
APO-1/Fas (CD95), a member of the TNF/NGF receptor superfamily, is a glycosylated 48 kDa surface protein containing a single transmembrane region. APO-1/Fas is expressed on a variety of human B and T cell lines, on many different tumor cells and on various normal human tissues. Triggering of APO-1/Fas by its ligand or by certain anti-APO-1/Fas monoclonal antibodies results in rapid induction of programmed cell death (Apoptosis) in susceptible cells. The tissue distribution of APO-1/Fas and of the APO-1/Fas ligand suggests that the APO-1/Fas receptor/ligand system plays an important role in various aspects of mammalian development and especially in the homeostasis of the immune system.
Expression of the APO-1/Fas cell surface protein is enhanced by IFN-γ and TNF and by activation in lymphocytes. APO-1/Fas also occurs in a soluble form (sAPO-1/Fas) devoid of a transmembrane region.
Elevated sAPO-1/Fas levels have been reported in sera from patients with high-and low-grade malignant B- and T-cell leukemias and systemic lupus erythematosus (Knipping et al., submitted). sAPO-1/Fas may prevent cells from undergoing APO-1/Fas ligand induced Apoptosis. Hence, secretion of sAPO-1/Fas may provide a mechanism for tumor cells to excape immunosurveillance and may be involved in leukemogenesis.

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 (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 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 sAPO-1/Fas. 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 (pg/ml)	Fluorescent Intensity (FI)
25000	232.4
8333	80.4
2778	30.3
926	11.6
309	4.8
103	2.3
34	1.4
0	0.8

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 sAPO-1/Fas 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 10 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.

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 sAPO-1/Fas (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 sAPO-1/Fas (see Table 2). It has been calculated to be 4.0%.
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 sAPO-1/Fas 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 sAPO-1/Fas	2.7	2.8	2.9	7.7	**4.0**

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 sAPO-1/Fas (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 sAPO-1/Fas, calculated on 12 determinations of each sample. It has been calculated to be 5.9%.
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 sAPO-1/Fas 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 sAPO-1/Fas	3.0	4.1	2.2	14.1	**5.9**

7.3 Specificity

Cross reactivity was tested with the human Th1/Th2 Multiplex Assay. There was no detectable cross reactivity observed.
(For detailed information refer to “Combination Table“ on www.eBioscience.com.)

7.4 Hook Effect

1:5 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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