PRODUCT INFORMATION & MANUAL

Human CD44var (v6) FlowCytomix Simplex Kit

BMS80210FF

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

eBioscience®
An Affymetrix Company

Human CD44var (v6) FlowCytomix Simplex Kit

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:
888.999.1371
858.642.2058
info@eBioscience.com

Fax:
858.642.2046

Europe/International*

Technical Support:
+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:
+43 1 796 40 40-304
europe@eBioscience.com

Fax:
+43 1 796 40 40-400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

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This human CD44var (v6) 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 CD44var (v6), Bead Population **A7**

2 vials human CD44var (v6) **Standard** (lyophilized): 2 µg/ml upon reconstitution

1 vial (350 µl) **Biotin-Conjugate** (20x) anti-human CD44var (v6) monoclonal antibody

2 INTENDED USE

BMS80210FF is a bead based Analyte Detection System for quantitative detection of human CD44var (v6) by Flow Cytometry. **BMS80210FF is for research use only. Not for use in diagnostic or therapeutic procedures.**

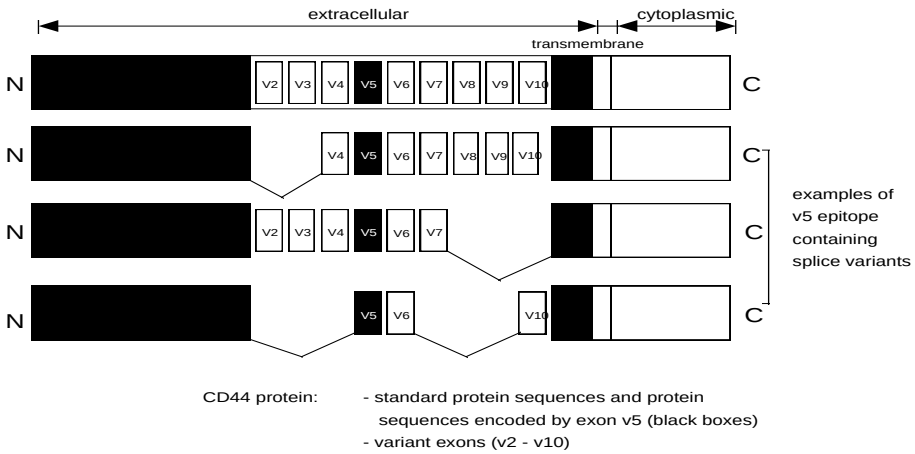
Please note: Samples must be **prediluted 1:30** in Assay Buffer (included in the Basic Kit BMS8420FF) before starting the test procedure.
In combination with other Simplex Kits it is recommended evaluating both, an undiluted and a 1:30 prediluted sample.

3 SUMMARY

CD44 (Pgp-1; Ly-24; ECMR III; F10-44-2; H-CAM; HUTCH-I; In(Lu)-related p80; Hermes antigen; hyaluronan receptor) is a polymorphic glycoprotein which participates in a wide variety of cell-cell or cell-matrix interactions including lymphocyte homing, establishment of B and T cell immune responses, tumor metastasis formation and inflammation.

Three isoform categories of the CD44 molecule have been identified:
1) a predominant 80-90 kDa category, the so-called standard form named C44std,
2) an intermediate size category of 110-160 kDa and
3) a category which includes very large isoforms of 250 kDa covalently modified by the addition of chondroitin sulfate.

This CD44-family of transmembrane receptor molecules is derived from a single gene located on chromosome 11. Alternative splicing of the mRNA gives rise to the different isoforms, containing inserts of varying sizes in the extracellular domain of the molecule (exons v2-v10). All CD44 isoforms are variably glycosylated. In contrast to standard CD44 (CD44std) which is almost ubiquitously expressed, the variety of CD44 isoforms (CD44var) have a much more restricted distribution, e.g., on keratinocytes (exons v3-v10), ephitelial cells (exons v8-v10), activated lymphocytes and macrophages (exon v6).
A splice variant of CD44 (exons v4-v7) confers metastatic behaviour in a rat carcinoma model; aberrant expression of splice variants has been detected on a variety of human tumor cell lines as well as primary and metastatic human tumors, including lymphomas, carcinomas (colon, thyroid, mamma, bladder), and gliomas. Detection of abnormal regulation of CD44 splicing thus could be helpful in cancer diagnosis and disease evaluation.
The sCD44var (v6) ELISA detects all circulating CD44 isoforms comprising the sCD44var (v6) sequences.



Determination of sCD44var (v6) will provide more detailed insight into different pathological modifications during cancer and other diseases.

- **brain tumors:** CD44 is strongly expressed in high-grade gliomas and weakly expressed in meningiomas, medulloblastomas and normal brain.

- **colorectal carcinomas:** in human colorectal neoplasia CD44 variant proteins are found on all invasive carcinomas and during carcinoma metastasis. Variants are already expressed at a relatively early stage of colorectal carcinogenesis and tumor progression.

- **gastric cancer:** tumors from patients suffering from stomach adenocarcinomas express CD44 variants. Adenocarcinomas of the intestinal type are strongly positive for exon v5 and v6, whereas diffuse type adenocarcinomas predominantly express exon v5.

- **lung, breast cancer:** in malignant tissues there is gross overproduction of alternatively-spliced large molecular variants in all samples, whereas in the control samples only the standard product was routinely detected with occasional minimal quantities of one or two small variants.

- **lymphoma:** in gastrointestinal lymphoma overexpression of CD44 has been correlated with poor survival and more disseminated disease.

Overexpression of CD44 is also found in several aggressive, but not low-grade, non-Hodgkin´s lymphomas as well as in Hodgkin´s and nodal diffuse lymphomas.

- **tonsil, skin cancer:** variant CD44 isoform expression can be demonstrated in the plasma membrane of squamous cells of skin and tonsil epithelial and is greatly diminished in malignant squamous epithelial tumors.

- **HIV:** CD44 is almost completely depleted from the surface of HIV-infected cells.

- **inflammatory joint diseases:** CD44 expression was decreased in synovial fluid neutrophils from most patients.

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) 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 CD44var (v6). 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)
100000	387.70
33333	186.24
11111	48.54
3704	10.27
1235	2.78
412	1.46
137	1.29
0	1.17

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 CD44var (v6) 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 126 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 CD44var (v6) (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 CD44var (v6) (see Table 2). It has been calculated to be 5.0%.
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 CD44var (v6) 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 CD44var (v6)	4.0	6.5	3.2	6.4	**5.0**

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 CD44var (v6) (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 CD44var (v6), calculated on 12 determinations of each sample. It has been calculated to be 5.8%.
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 CD44var (v6) 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 CD44var (v6)	3.7	6.3	4.8	8.6	**5.8**

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

1:30 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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Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

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877.726.8559
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858.642.2046

Europe/International*

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+43 1 796 40 40-120
tech@eBioscience.com

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+43 1 796 40 40-304
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