

PRODUCT INFORMATION AND MANUAL

***FlowCytomix
Mouse CXCL1/KC Simplex Kit***

BMS86019FF

For research use only.

Not for diagnostic or therapeutic procedures.

96 Tests

**Mouse CXCL1/KC
BMS86019FF Simplex Kit**



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This mouse CXCL1/KC Simplex Kit must be used in combination with FlowCytomix mouse/rat Basic Kit BMS8440FF. For test procedure, measurement and calculation of results please refer to FlowCytomix mouse/rat Basic Kit BMS8440FF manual.

1 REAGENTS PROVIDED

- 1 vial (175 μ l) **Fluorescent Beads** (20x) coated with monoclonal antibody to mouse CXCL1/KC, Bead Population **A5**
- 2 vials mouse CXCL1/KC **Standard** (lyophilized): 70 ng/ml upon reconstitution
- 1 vial (350 μ l) **Biotin-Conjugate** (20x) anti-mouse CXCL1/KC polyclonal antibody

2 INTENDED USE

BMS86019FF is a bead based Analyte Detection System for quantitative detection of mouse CXCL1/KC by Flow Cytometry. **BMS86019FF is for research use only. Not for use in diagnostic or therapeutic procedures.**

3 SUMMARY

The KC (Keratinocyte chemoattractant) gene is a member of the small inducible gene family of regulated chemokines. It encodes a growth factor and chemoattractant that appears to mediate inflammatory and immune responses *in vitro* and *in vivo*.

Chemokine KC (CXCL1) has been considered to be a murine homologue of human GRO/MGSA and was identified as chemoattractant for monocytes and neutrophils. The mouse KC gene is an alpha-chemokine gene whose transcription is induced in mononuclear phagocytes by LPS. Transcriptional control of the KC gene is distinct from that of the three human GRO genes and the mouse MIP-2 gene.

The mouse KC cDNA encodes a 96 amino acid residue precursor protein from which the amino-terminal 19 amino acid residues are cleaved to generate the 77 amino acid residue mature KC. The protein sequence of mouse KC shows approximately 63% identity to that of mouse MIP-2, another mouse alpha chemokine. In addition, the protein sequence of KC is approximately 60% identical to the human GROs. Like other alpha chemokines, mouse KC is a potent neutrophil attractant and activator. Moreover, keratinocyte chemoattractant (KC)/ growth-regulated oncogene (GRO) chemokines and pro-inflammatory chemokines network in mouse.

The activities of KC and MIP-2 have been shown to be mediated by the unique mouse IL-8 receptor that shows 71% and 68% amino acid sequence identity to human IL-8R and IL-8R, respectively. Since an IL-8 homologue has not been identified in mice, it has been suggested that MIP-2 and KC are the functional homologues of IL-8 and may function as the major proinflammatory alpha chemokines in mice.

Increased KC expression has been found to be associated with neutrophil influx in various inflammatory conditions. An important role for KC/CXCL1 has been shown in experimental model of multiple sclerosis as a chemoattractant of destructive immune cells. Data further indicate that KC/GRO chemokines are the principal chemokines induced by LPS and pro-inflammatory cytokines IL-1 and TNF via NFkappaB signalling in ovarian surface epithelial cancer cells.

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

Plasma (citrate) was tested with this assay. Other biological samples might be suitable for use in the assay. Remove plasma from cells as soon as possible after 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 mouse CXCL1/KC. 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)
3500	513.15
1167	140.95
389	32.92
130	8.42
43	3.79
14	2.43
5	2.13
0	1.97

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 mouse CXCL1/KC 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 3.0 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 mouse CXCL1/KC (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 mouse CXCL1/KC (see Table 2). It has been calculated to be 6.6%.

Individual user data may vary due to differences in protein content of plasma pools or individual donor plasma.

Table 2

The coefficient of variation of the mouse CXCL1/KC 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 (%)
m CXCL1/KC	4.2	5.5	7.5	9.2	6.6

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 mouse CXCL1/KC (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 mouse CXCL1/KC, calculated on 12 determinations of each sample. It has been calculated to be 8.2%.

Individual user data may vary due to differences in protein content of plasma pools or individual donor plasma.

Table 3

The coefficient of variation of the mouse CXCL1/KC 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 (%)
m CXCL1/KC	7.7	8.3	4.0	12.8	8.2

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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