## invitrogen

PI AHC1171

## Mouse (monoclonal) Anti-Human Fractalkine (CX3C) Unconjugated

## **PRODUCT ANALYSIS SHEET**

Catalog Number:	AHC1171
Lot Number:	See product label
Quantity/Volume:	100 μg/0.075 mL
Clone Number:	KH-12
Isotype:	IgGlκ
Form of Antibody:	Purified immunoglobulin in borate buffered saline, pH 8.0.
Preservation:	Preservative-free.
Purification:	Purified by Protein A affinity chromatography.
Immunogen:	The complete human fractalkine sequence expressed in E. coli.
Myeloma/Fusion Partners:	Produced by the fusion between BALB/c splenocytes and mouse myeloma Sp2/0-Ag14 cells.
Specificity:	Fractalkine is a newly discovered chemokine that has the unusual CX3C amino acid residue motif. Members of the CX3C family include only fractalkine and its murine homologue, neurotactin. Fractalkine exists in two forms: membrane-bound and soluble. The membrane-bound version is composed of a transmembrane domain, an elongated mucin-like domain, and the chemokine related domain. This membrane-bound form (373 amino acid residues) has $M_r$ =105 kDa. Fractalkine's soluble form (317 amino acid residues) has $M_r$ =95 kDa. Fractalkine, therefore, is a veritable giant among the chemokines, whose molecular masses typically range from 7.5 to 15 kDa. Both the soluble and membrane-bound forms exert their actions through the fractalkine receptor, CX3CR1, a receptor, which was formerly known as the orphan receptor V28. Fractalkine binding to its receptor enhances [gamma- <sup>35</sup> S] guanine triphosphate binding to cell membranes, implicating receptor coupling to G proteins, and produces a transient Ca <sup>2+</sup> influx. Both the soluble and the membrane-bound forms of fractalkine are potent chemoattractants for T-cells, monocytes, and IL-2 activated NK cells, three types of leukocytes that express the fractalkine receptor. In addition to inducing migration of these cells, the membrane-associated form expressed by activated endothelium promotes leukocyte/endothelium adhesion. Fractalkine is constitutively expressed by neurons, while its expression in astroglia is under the regulation of TNF-α and IL-1β. Microglia express CX3CR1. The interaction of the fractalkine borne on the neurons with the CX3CR1 borne on the microglia mediates adhesion between these two cell types. This interaction is probably crucial to the cytokine-regulated activation of microglia by astroglia.

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Specificity (cont'd):	Recognizes natural and recombinant human fractalkine.
Species Reactivity:	Human and rat. Other species were not tested.
Applications:	This antibody is suitable for use in immunoprecipitation, flow cytometry, Western blotting and ELISA.
Suggested Working Dilutions:	The recommended concentration for Western blotting is 0.5-1.0 $\mu$ g/mL. For flow cytometry, 0.5-1.5 $\mu$ g per test is recommended. The optimal concentration should be determined for each specific application.
Storage:	Store at $-20^{\circ}$ C. Upon initial thawing, apportion into working aliquots store at $-20^{\circ}$ C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody.
References:	Bazan, J.F. et al. (1997) A new class of membrane-bound chemokine with a CX3C motif. Nature 385(6617):640-644.
	Harrison, J.K. et al. (1998) Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc. Nat'l. Acad. Sci. (USA) 95(18):10896-10901.



Proteins were resolved from PC12 cells (rat pheochromocytoma) by SDS-PAGE and transferred to PVDF membrane. The membrane was incubated with 1  $\mu$ g/mL of the anti-fractalkine antibody. The signal was detected using a Goat F(ab')<sub>2</sub> anti-Mouse IgG alkaline phosphatase antibody (cat. #AMI4405) at a 1:5000 dilution and the membrane was incubated with CDP-substrate using the Westernstar<sup>TM</sup> method (Tropix). The membrane was then exposed to Kodak BioMax film for 1 minute.

This product is for research use only. Not for use in diagnostic procedures.

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