Rat CX3CL1/Fractalkine Chemokine Domain Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF537

DESCRIPTION

Species Reactivity	Rat
Specificity	Detects rat CX3CL1/Fractalkine in ELISAs and Western blots. In Western blots, less than 5% cross-reactivity with recombinant human (rh) Eotaxin is observed. Neutralizes the biological activity of rhCX3CL1 and rmCX3CL1 with similar effectiveness.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant rat CX3CL1/Fractalkine Gln25-Gly100 Accession # O55145
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

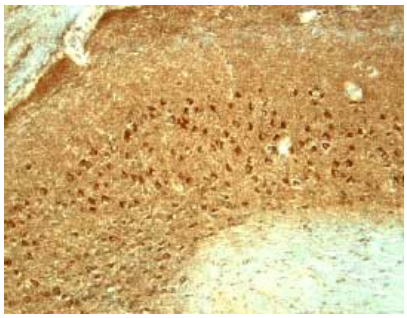
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	Recombinant Rat CX3CL1/Fractalkine (Catalog # 536-FR)
Immunohistochemistry	5-15 µg/mL	See Below
Rat CX3CL1/Fractalkine Sandwich Immunoassay		**Reagent**
ELISA Capture	0.2-0.8 µg/mL	Rat CX3CL1/Fractalkine Chemokine Domain Antibody (Catalog # AF537)
ELISA Detection	0.1-0.4 µg/mL	Rat CX3CL1/Fractalkine Chemokine Domain Biotinylated Antibody (Catalog # BAF537)
Standard		Recombinant Rat CX3CL1/Fractalkine (Catalog # 536-FR)
Neutralization	Measured by its ability to neutralize CX3CL1/Fractalkine-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CX3CR1. The Neutralization Dose (ND_{50}) is typically 0.3-1.2 µg/mL in the presence of 40 ng/mL Recombinant Rat CX3CL1/Fractalkine.	

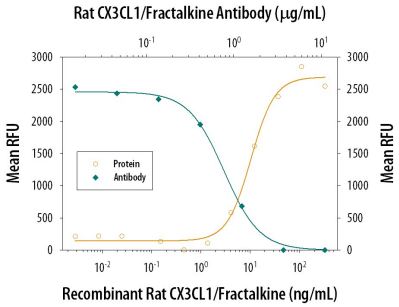
DATA

Immunohistochemistry



CX3CL1/Fractalkine in Mouse Brain.
CX3CL1/Fractalkine was detected in perfusion fixed frozen sections of mouse brain (cingulate cortex) using 1.7 µg/mL Goat Anti-Rat CX3CL1/Fractalkine Chemokine Domain Antigen Affinity-purified Polyclonal Antibody (Catalog # AF537) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Neutralization



Chemotaxis Induced by CX3CL1/Fractalkine and Neutralization by Rat CX3CL1/Fractalkine Antibody.
Recombinant Rat CX3CL1/Fractalkine (Catalog # 536-FR) chemoattracts the BaF3 mouse pro-B cell line transfected with human CX3CR1 in a dose-dependent manner (orange line). The amount of cells that migrated through to the lower chemotaxis chamber was measured by Resazurin (Catalog # AR002). Chemotaxis elicited by Recombinant Rat CX3CL1/Fractalkine (40 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Rat CX3CL1/Fractalkine Chemokine Domain Antigen Affinity-purified Polyclonal Antibody (Catalog # AF537). The ND_{50} is typically 0.3-1.2 µg/mL.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

CX3CL1, also named neurotactin, is a member of the delta chemokine subfamily that contains a novel C-X_3-C motif. Unlike other known chemokines, CX3CL1 is a type 1 membrane protein containing a chemokine domain tethered on a long mucin-like stalk. Rat CX3CL1 cDNA encodes a 393 amino acid (aa) residue precursor protein with two alternative (21 aa or 24 aa residue) putative signal peptides, a 74 aa or 76 aa residue globular chemokine domain, a 238 aa residue stalk region rich in Gly, Pro, Ser and Thr and containing degenerate mucin-like repeats, a 19 aa residue transmembrane segment and a 36 aa residue cytoplasmic domain. The extracellular domain of CX3CL1 can potentially be released as a soluble protein by proteolysis at the conserved dibasic motif proximal to the transmembrane region. With the exception of the stalk region, rat CX3CL1 shares a high degree of amino acid sequence homology (83% sequence identity) with human and mouse CX3CL1. CX3CL1 is expressed in various tissues including heart, brain, lung, kidney, skeletal muscle, and testis. In rat brain, CX3CL1 expression was found to be localized principally to neurons. The expression of CX3CL1 was also reported to be up-regulated on activated endothelial cells. Membrane-bound CX3CL1 has been shown to promote adhesion of leukocytes. The soluble chemokine domain of human CX3CL1 was reported to be chemotactic for T cells and monocytes while the soluble chemokine domain of mouse CX3CL1 was reported to chemoattract neutrophils and T-lymphocytes but not monocytes. CX3CR1, previously named V28 or chemokine beta receptor-like 1, has been found to be a specific receptor for CX3CL1. In addition, US28, a 7TM receptor encoded by human cytomegalovirus that binds multiple CC chemokines, has also been shown to bind CX3CL1 with high-affinity.

References:

1. Kledal, T.N. *et al.* (1998) FEBS Lett. **441**:209.
2. Combadiere, C. *et al.* (1998) J. Biol. Chem. **273**:23799.
3. Harrison, J.L. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:10896.
4. Rossi, D.L. *et al.* (1998) Genomics **47**:163.

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