eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ CX_3CR1 Chemokine Membrane Preparation

CATALOG NUMBER:	HTS015M	**QUANTITY:**	200 units
LOT NUMBER:	SC260679	**VOLUME/CONCENTRATION:**	1 mL, 0.5 mg/mL

BACKGROUND:

CX_3CR1 is a GPCR expressed on natural killer cells, cytotoxic T lymphocytes, and macrophages. The sole ligand for CX_3CR1, fractalkine, is an unusual chemokine that is expressed as a transmembrane molecule with a CX3C domain and a mucin domain (Imai *et al*., 1997). Fractalkine is highly expressed on endothelial cells activated by TNFα and other proinflammatory cytokines, and fractalkine/CX_3CR1 interactions mediate recruitment of macrophages into the atherosclerotic plaque (Lesnick *et al*., 2003; McDermott *et al*., 2003). In addition, fractalkine and CX_3CR1 have been implicated in the pathogenesis of glomerulonephritis, HIV infection, and rheumatoid arthritis (Ito *et al*., 2002; Faure *et al*., 2003; Nanki *et al*., 2002). CX_3CR1 membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression. Thus, they are ideal HTS tools for screening of antagonists of CX_3CR1 interactions with fractalkine. The membrane preparations exhibit a Kd of 0.21 nM for [^{125}I]-Fractalkine. With 2.5 μg/well of CX_3CR1 Membrane Prep and 0.05 nM [^{125}I]-Fractalkine, a greater than 5-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand Binding Assay

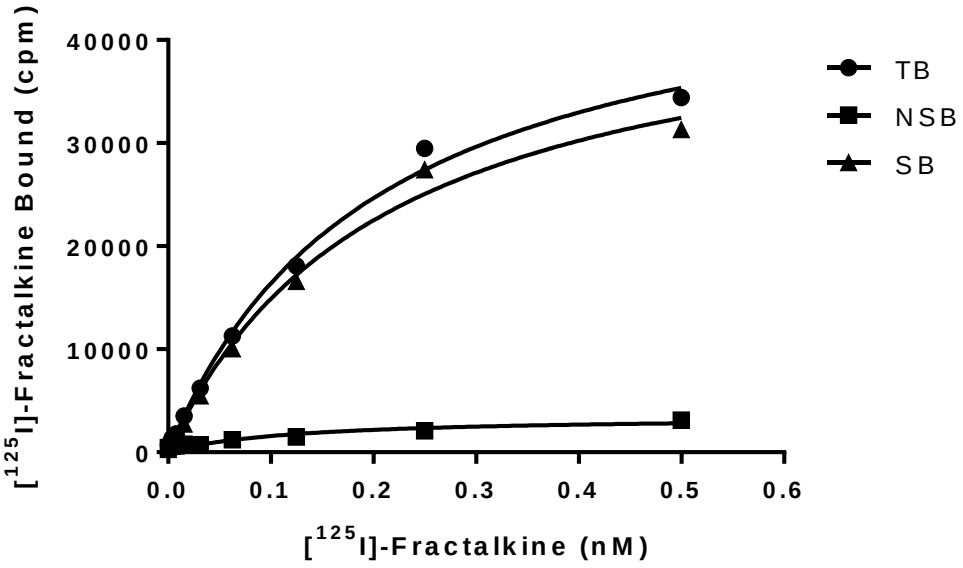

Figure 1. Saturation Binding for CX_3CR1. 2.5 μg/well of CX_3CR1 Membrane Preparation were incubated with increasing amounts of [^{125}I]-Fractalkine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled human recombinant fractalkine. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC260679.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

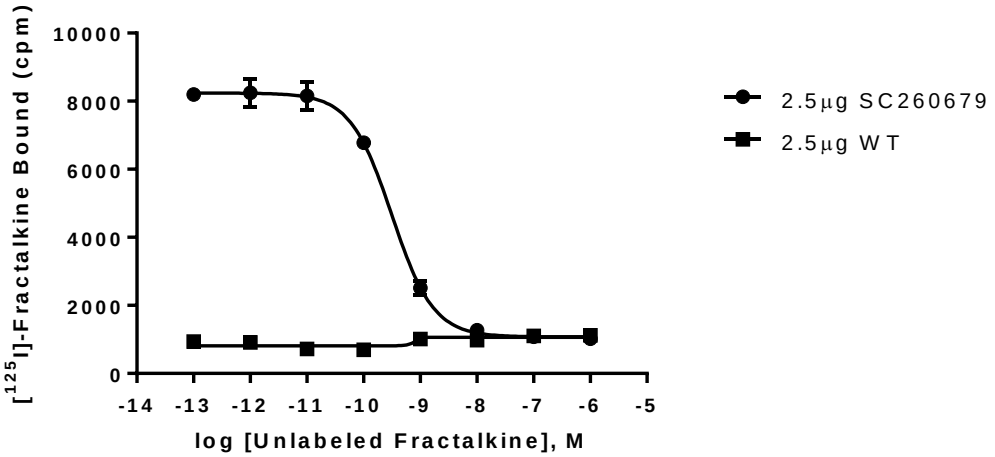

Figure 2. Competition Binding for CX_3CR1. 2.5 µg/well of CX_3CR1 Membrane Preparation or Wild-Type Chem-1 Membrane Preparation (WT; Catalog # HTS000MC1) were incubated with 0.05 nM [^{125}I]-Fractalkine and increasing concentrations of unlabeled fractalkine, and more than a 5-fold signal:background ratio was obtained. The data are from a representative sample of lot SC260679.

SPECIFICATIONS: 1 unit = 2.5 µg
B_{max} for [^{125}I]-Fractalkine Binding: 7.5 pmol/mg protein
K_d for [^{125}I]-Fractalkine Binding: 0.21 nM
Signal:Background: ≥5-fold

SPECIES: Human CX_3CR1 (Accession number U28934)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CX_3CR1 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate and incubated for 2 h at room temperature. Prior to filtration, a GF/C 96-well filter plate was coated with 0.33% polyethyleneimine for 30 min and then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions were transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted for determination of receptor-associated radioligand binding.

Binding Buffer: 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{125}I]-Fractalkine (PerkinElmer # NEX368)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than a 5-fold signal:background ratio with [^{125}I]-Fractalkine at 0.05 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris, pH 7.4, 10% glycerol, and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to 0.5 mg/mL in packaging buffer, dispensed at 1 mL per vial, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Faure S *et al*. (2003) Deleterious genetic influence of CX3CR1 genotypes on HIV-1 disease progression. *J Acquir. Immune Defic. Syndr.* 32:335-7.
2. Imai T *et al.* (1997) Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 91:521-30.
3. Ito Y *et al*. (2002) Fractalkine expression and the recruitment of CX3CR1+ cells in the prolonged mesangial proliferative glomerulonephritis. *Kidney Int.* 61:2044-57.
4. Lesnick P *et al*. (2003) Decreased atherosclerosis in CX3CR1-/- mice reveals a role for fractalkine in atherogenesis. *J. Clin. Invest*. 111:333-40.
5. McDermott DH *et al.* (2003) Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. *J. Clin. Invest.* 111:1241-50.
6. Nanki T *et al.* (2002) Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheum.* 46:2878-83.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.