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PRODUCT DATASHEET

ChemiScreen™ CXCR1 Chemokine Membrane Preparation

CATALOG NUMBER:	HTS001M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

CXCR1 (also known as IL-8RA) is a Gi/o-coupled GPCR expressed on neutrophils, CD8(+) T cells, and intestinal epithelial cells (Murphy, 1997; Sturm *et al*., 2005; Takata *et al*., 2004). CXCR1 binds specifically to the chemokine interleukin-8 (IL-8), whereas a related receptor, CXCR2, binds promiscuously to IL-8 and several other chemokines. CXCR1 has been proposed to be the primary regulator of neutrophil response to IL-8 in sepsis (Cummings *et al*., 1999). Antagonists of CXCR1 and CXCR2 have been developed and display efficacy in animal models of reperfusion injury (Bertini, *et al*., 2004; Kaneider *et al*., 2005). CXCR1 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 CXCR1 interaction with IL-8. The membrane preparations exhibit a Kd of 0.2 nM for [^{125}I]-IL-8. With 5 μg/well CXCR1 Membrane Prep and 0.1 nM [^{125}I]-IL-8, a greater than 5-fold signal-to-background ratio is obtained.

APPLICATIONS:

Radioligand binding assay

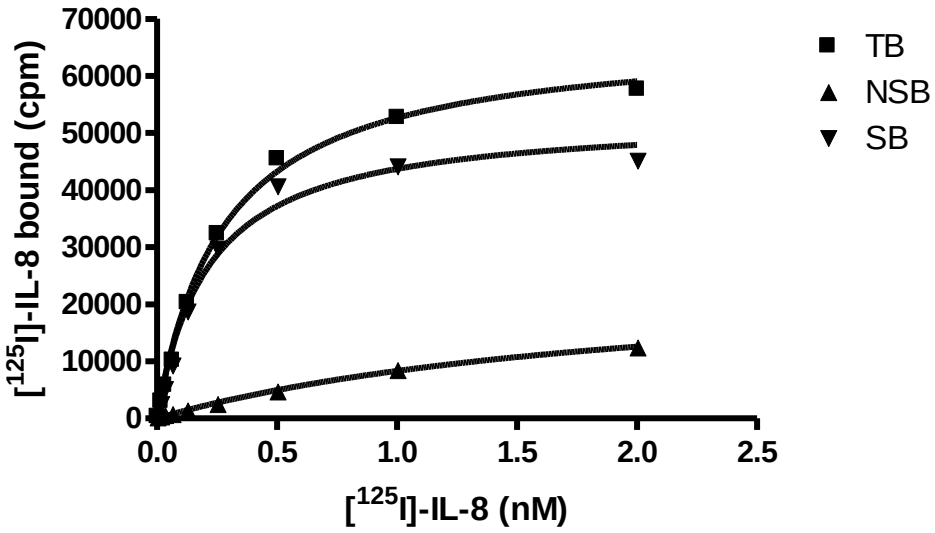

Figure 1. Saturation binding for CXCR1. 10 μg/well CXCR1 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled Interleukin-8 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Interleukin-8. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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

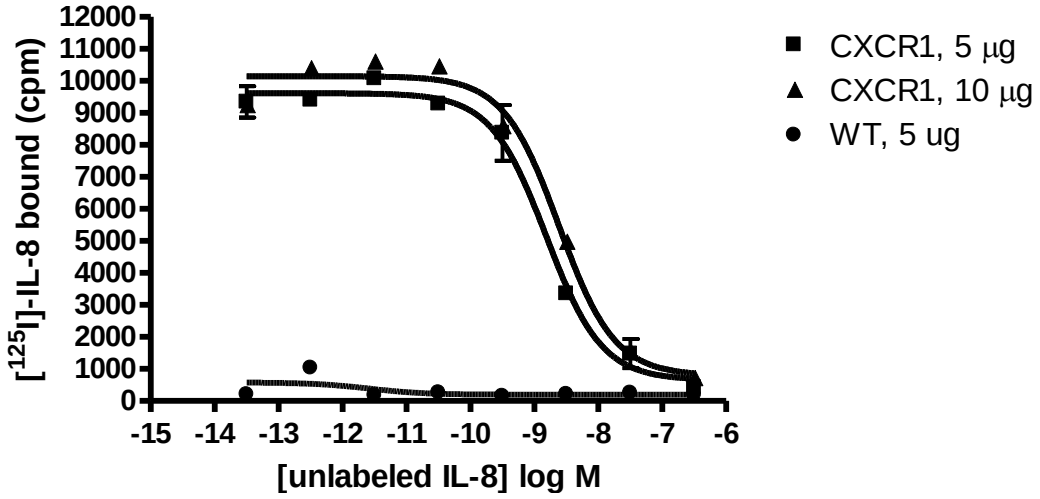

Figure 2. Competition binding for CXCR1. CXCR1 Membrane Preparation at 5 and 10 µg/well and Wild-Type-Chem-1 Membrane Preparation (WT, cat. # HTS000MC1) were incubated with 0.1 nM ^{125}I-labeled Interleukin-8 and increasing concentrations of unlabeled Interleukin-8. With CXCR1 Membrane Preparation and unlabeled IL-8, more than 5-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with CXCR1 membrane preparation in a dose response curve with unlabeled IL-8.

	5 µg/well	10 µg/well
Signal:background	14.8	12.8
Specific binding (cpm)	8965	9624

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 3.7 pmol/mg
K_d: 0.2 nM

Species: Human CXCR1 Receptor (Accession number M68932)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CXCR1 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

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] Interleukin-8 (Perkin Elmer #NEX277)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM 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 5-fold signal:background with ^{125}I-labeled IL-8 at 0.1 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 the indicated concentration in 1 ml packaging buffer, 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. Do not freeze and thaw.

REFERENCES:

1. Bertini R *et al.* (2004) Noncompetitive allosteric inhibitors of the inflammatory chemokine receptors CXCR1 and CXCR2: prevention of reperfusion injury. *Proc. Natl. Acad. Sci. USA* 101(32):11791-6.

2. Cummings CJ *et al.* (1999) Expression and function of the chemokine receptors CXCR1 and CXCR2 in sepsis. *J. Immunol.* 162(4):2341-6.

3. Kaneider NC *et al.* (2005) Reversing systemic inflammatory response syndrome with chemokine receptor pepducins. *Nat Med.* 11(6):661-5.

4. Murphy PM (1997) Neutrophil receptors for interleukin-8 and related CXC chemokines. *Semin. Hematol.* 34: 311-8.

5. Sturm A *et al.* (2005) CXCL8 modulates human intestinal epithelial cells through a CXCR1 dependent pathway. *Cytokine* 29(1):42-8.

6. Takata H *et al.* (2004) Cutting edge: expression of chemokine receptor CXCR1 on human effector CD8+ T cells. *J. Immunol.* 173(4):2231-5.

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