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PRODUCT DATASHEET

ChemiScreen™ CXCR3 Membrane Preparation

CATALOG NUMBER:	HTS003M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

CXCR3 is a 7-TM GPCR that is selective for the CXC chemokines IP10, ITAC and MIG (Loetscher *et al.*, 1996). Binding of IP10 and MIG to CXCR3 induces Ca^{2+} mobilization, chemotaxis and inflammatory responses of T lymphocytes, and also act as potent inhibitors of angiogenesis. CXCR3 is highly expressed in IL-2-activated T lymphocytes in vitro (Loetscher *et al.*, 1996) and in T lymphocytes present in inflamed tissues in rheumatoid arthritis and multiple sclerosis (Balashoy *et al.*, 1999; Qin *et al.*, 1998). In vivo, neutralization of CXCR3 inhibits experimentally induced type I diabetes (Frigerio *et al.*, 2002), peritonitis (Xie *et al.*, 2003), and post-lung transplantation bronchiolitis obliterans syndrome (Belperio *et al.*, 2002). ChemiScreen™ CXCR3 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 agonists of CXCR3 receptor interactions. The membrane preparations exhibit a K_d of 2.6 nM for [^{125}I]-IP-10. With 5 μg/well of CXCR3 Membrane Prep and 0.1 nM [^{125}I]-IP-10, a greater than 10-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand Binding Assay

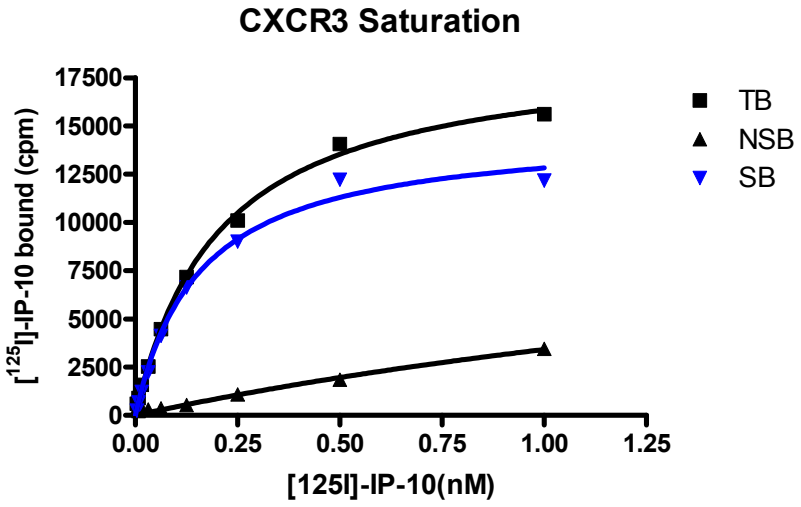

Figure 1. Saturation Binding for CXCR3. 5 μg/well of CXCR3 Membrane Preparation were incubated with increasing amounts [^{125}I]-IP-10 in the absence (TB: total binding) or presence (NSB: nonspecific binding) of a 200-fold excess of unlabeled IP-10. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC.

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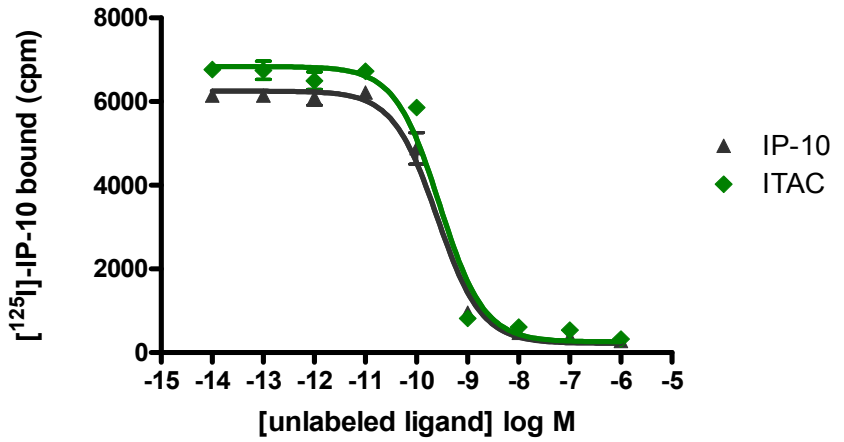

Figure 2. Competition Binding for CXCR3. 5 μg/well of CXCR3 Membrane Preparation or Wild-Type Chem-1 Membrane Preparation (Catalog # HTS000MC1) were incubated with 0.1 nM [^{125}I]-IP-10 and increasing concentrations of unlabeled IP-10, and more than a 10-fold signal:background ratio was obtained. The data are from a representative sample of lot SC.

SPECIFICATIONS: 1 unit = 5 μg membrane preparation
B_{max} for [^{125}I]-IP-10 Binding: 0.6 pmol/mg protein
K_d for [^{125}I]-IP-10 Binding: ~0.2 nM
Signal:Background: ≥10-fold

TRANSFECTION: Full-length human CXCR3 cDNA (Accession Number: X95876).

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CXCR3 receptor 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, an FC 96-well harvest plate was coated with 0.33% polyethyleneimine for 30 min, 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]-IP-10 (PerkinElmer # NEX348)

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 10-fold signal:background ratio with [^{125}I]-IP-10 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 1 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. Balashov KE, *et al.* (1999) CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1α and IP-10 are expressed in demyelinating brain lesions.*Proc. Natl. Acad. Sci. USA* 96:6873-8.

2. Belperio JA, *et al.* (2002) Critical role for CXCR3 chemokine biology in the pathogenesis of bronchiolitis obliterans syndrome. *J. Immunol.* 169:1037-1049.

3. Frigerio S, *et al.* (2002) Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nat. Med.* 8:1414-20.

4. Loetscher M, *et al*. (1996) Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes.*J Exp Med.* Sep 1; 184(3):963-9.

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6. Xie JH, *et al.* (2003) Antibody-mediated blockade of the CXCR3 chemokine receptor results in diminished recruitment of T helper 1 cells into sites of inflammation.*J. Leukoc. Biol.* 73:771-7-80.

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