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Chemiscreen™ MEMBRANE PREPARATION
HUMAN RECOMBINANT CXCR2 CHEMOKINE RECEPTOR

CATALOG NUMBER:	HTS002M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	2 mL, 0.5 mg/mL

BACKGROUND:

CXCR2 is a 7-TM G-protein coupled receptor that binds to the chemokines GROα, GROβ, GROγ, IL-8, ENA-78, NAP-2 and GCP-2 (Olson and Ley, 2002). Neutrophils, mast cells and microvascular endothelial cells express CXCR2, and interactions of CXCR2 with its ligands promotes chemotaxis of these cell types (Heidemann *et al*., 2003; Nilsson *et al*., 1999; White *et al*., 1998). Studies with mice lacking CXCR2 indicate that CXCR2 promotes growth of primary tumors and secondary metastases (Keane *et al*., 2004), and plays an essential role in hyperoxia-induced lung injury (Sue *et al*., 2004). In addition, cytomegalovirus encodes a CXCR2-binding chemokine, vCXC-1, that promotes neutrophil migration to infected cells (Penfold *et al*., 1999). Chemicon's CXCR2 Membrane Preps are ideal tools for screening for antagonists of interactions between CXCR2 and its ligands. Radiolabeled IL-8 binds to the CXCR2 membranes with a Kd of 0.075 nM. With 0.1 nM ^{125}I-labeled IL-8 and 5 μg CXCR2 membranes, an 8-fold window of specific binding is obtained.

APPLICATIONS:

Radioligand binding assay

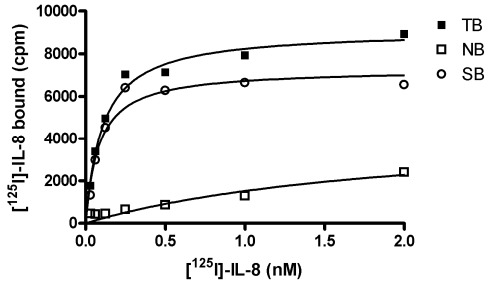

Figure 1. Saturation binding for CXCR2. 10 μg/well of CXCR2 Membrane Preparation was incubated with [^{125}I]-IL-8 in the absence (TB=total binding) or presence (NSB=nonspecific binding) of a 200-fold excess of unlabeled IL-8. The CXCR2 Membrane Prep binds specifically to ^{125}I-labeled ligand IL-8, with K_d of 0.075 nM

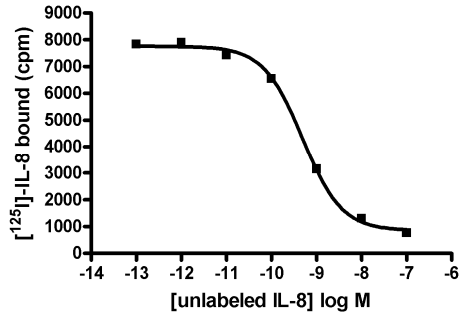

Figure 2. Competition binding for CXCR2. 5 μg/well CXCR2 Membrane Preparation was incubated with 0.1 nM ^{125}I-labeled IL-8 and increasing concentrations of unlabeled IL-8, and more than 8-fold signal:background was obtained.

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Table 1. Specific binding and signal:background in competition radioligand binding assay with CXCR2 membrane preparation

	5.0 μg
Specific Binding	7059
Signal:Background	10.3

SPECIFICATIONS: 1 unit = 5 μg membrane prep
Bmax for [^{125}I]IL-8 binding: 0.93 pmol/mg protein
Kd for [^{125}I] IL-8 binding: ~ 0.075 nM

HOST CELLS: Chem-1

SPECIES: Full-length human CXCR2 cDNA (Accession Number: M73969)

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, a GF/C 96-well filter plate 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] IL-8 (Perkin Elmer# NEX277)

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

One package contains 200 units, where a unit is the amount of membrane that will yield greater than 8-fold signal:background with 0.1 nM ^{125}I-labeled IL-8.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives. Packaging method: Membranes protein were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Maintain frozen at –70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Heidemann J, *et al.* (2003) Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. *J. Biol. Chem.* 278: 8508-15

Keane MP, *et al.* (2004) Depletion of CXCR2 inhibits tumor growth and angiogenesis in a murine model of lung cancer. *J. Immunol.* 172: 2853-60.

Nilsson G, *et al.* (1999) Mast cell migratory response to interleukin-8 is mediated through interaction with chemokine receptor CXCR2/Interleukin-8RB. *Blood* 93: 2791-7

Olson TS and Ley K (2002) Chemokines and chemokine receptors in leukocyte trafficking.

Am. J. Physiol. Regul. Integr. Comp. Physiol. 283: R7-28

Penfold ME, *et al.* (1999) Cytomegalovirus encodes a potent alpha chemokine. *Proc Natl Acad Sci USA* 96: 9839-44

Sue RD, *et al.* (2004) CXCR2 is critical to hyperoxia-induced lung injury. *J. Immunol.* 172: 3860-8

White JR, *et al.* (1998) Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. *J. Biol. Chem.* 273: 10095-8

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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