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# PRODUCT DATASHEET

## ChemiScreen™ CXCR2 Chemokine Membrane Preparation

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|---|---|---|---|
| **CATALOG NUMBER:** | HTS002M | **QUANTITY:** | 200 units |
| **LOT NUMBER:** | JH1931155 | **VOLUME/CONCENTRATION:** | 1 mL, 1 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). EMD Millipore's CXCR2 Membrane Preps are ideal tools for screening for antagonists of interactions between CXCR2 and its ligands.

**APPLICATIONS:**

Radioligand Binding Assay

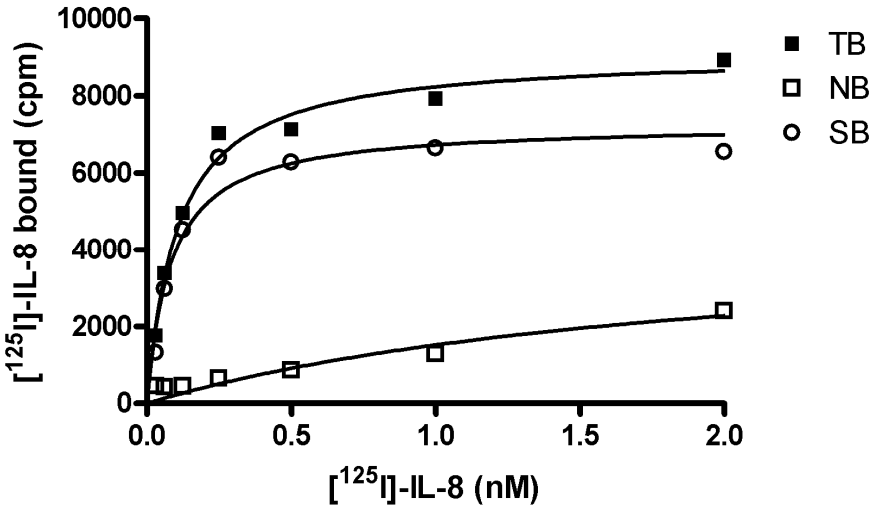


**Figure 1. Saturation binding for CXCR2.** 10 μg/well of CXCR2 Membrane Preparation was incubated with [$^{125}$I]-IL-8 in the absence (TB=total binding) or presence (NSB=nonspecific binding) of a 200-fold excess of unlabeled IL-8. The CXCR2 Membrane Prep binds specifically to $^{125}$I-labeled ligand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


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Template version: 7. Modified Date: 21NOV18. Modified by: JVK

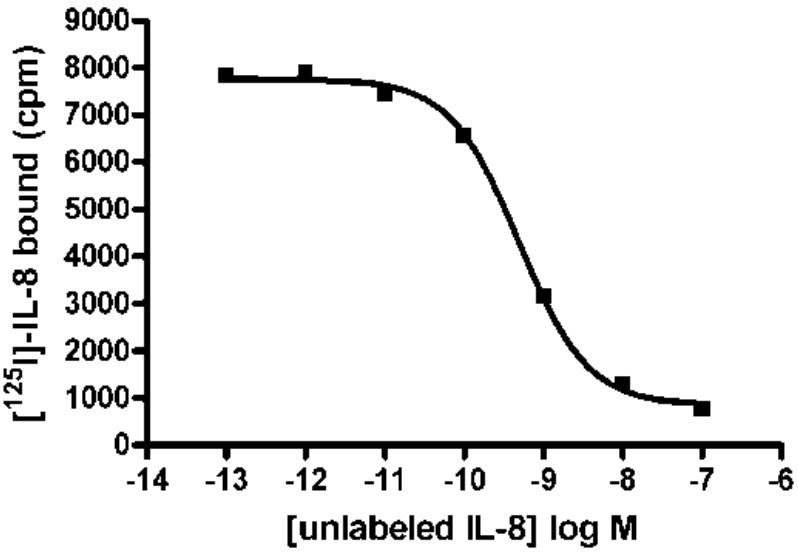


**Figure 2. Competition binding for CXCR2.** 5 μg/well CXCR2 Membrane Preparation was incubated with 0.1 nM $^{125}$I-labeled IL-8 and increasing concentrations of unlabeled IL-8, and more than 8-fold signal:background was obtained. Representative sample data.

**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
Signal:background: ≥8-fold

**TRANSFECTION:** 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 MgCl2, 1 mM CaCl2, 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:** 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. 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
2. 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.
3. 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
4. Olson TS and Ley K (2002) Chemokines and chemokine receptors in leukocyte trafficking. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283: R7-28
5. Penfold ME, et al. (1999) Cytomegalovirus encodes a potent alpha chemokine. Proc Natl Acad Sci USA 96: 9839-44
6. Sue RD, et al. (2004) CXCR2 is critical to hyperoxia-induced lung injury. J. Immunol. 172: 3860-8
7. 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


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