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PRODUCT DATASHEET

ChemiScreen™ CXCR4 Chemokine Membrane Preparation

CATALOG NUMBER:	HTS004M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION**	1 mL, 1 mg/mL

BACKGROUND:

The chemokine SDF-1α and its GPCR receptor CXCR4 have a one-to-one specificity that is unique among chemokines and their receptors. SDF-1α binds to CXCR4 expressed on hematopoietic and lymphopoietic cells, and directs their trafficking to and retention in hemato-and lymphatopoietic organs and sites of inflammation (Kucia *et al*., 2004). CXCR4 is expressed on several tumor cell lineages, and might be responsible for metastasis to sites of SDF-1α expression, such as bone and lymph nodes (Muller *et al*., 2001). In addition, CXCR4 is a coreceptor for the HIV envelope glycoprotein gp120 (Feng *et al*., 1996). Small molecule antagonists of CXCR4 have been developed and shown to inhibit infectivity of T-tropic HIV strains and to impair growth of brain tumors (Arakaki *et al*., 1999; Rubin *et al*., 2003). CXCR4 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 tools for screening for antagonists of interactions between CXCR4 and SDF-1α. [^{125}I]-SDF-1α binds to the CXCR4 membranes with a Kd of 0.9 nM. With 0.13 nM [^{125}I]-SDF-1α and 5 μg/well of CXCR4 membrane preparation, a greater than 4-fold signal to background ratio is obtained.

APPLICATIONS:

Radioligand Binding Assay

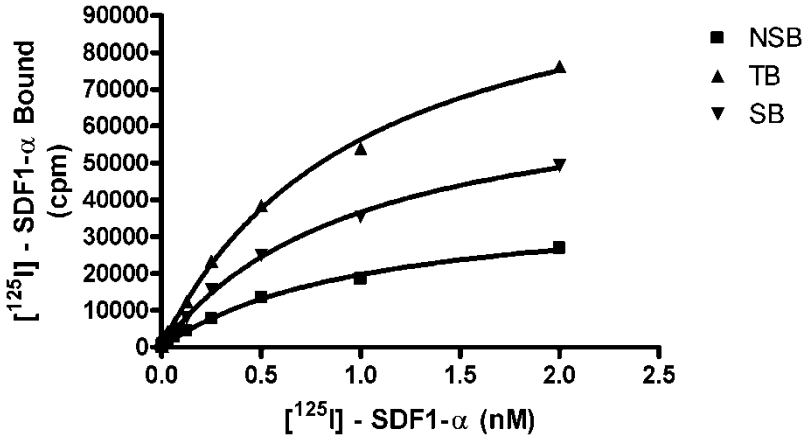

Figure 1. Saturation Binding for CXCR4. 5 μg/well of CXCR4 Membrane Preparation was incubated with increasing amounts of [^{125}I]-SDF-1α in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled SDF-1α. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative lot.

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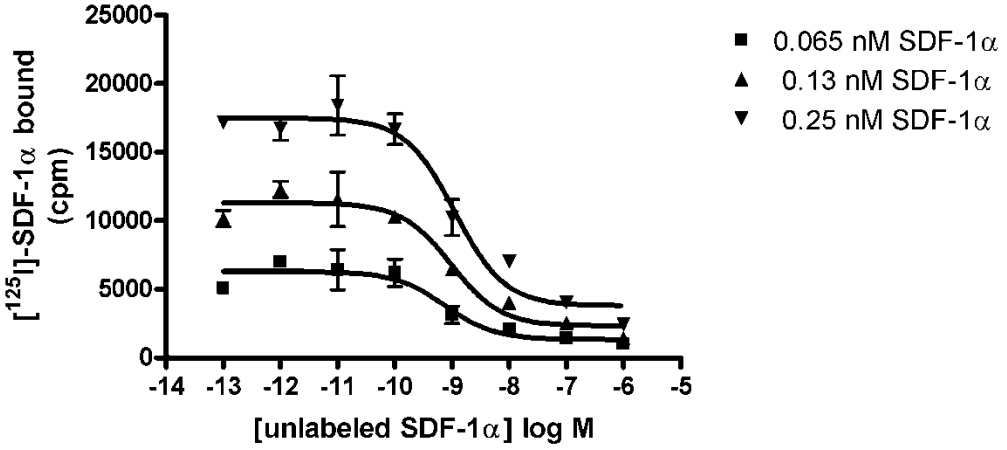

Figure 2. Competition Binding for CXCR4. 5 µg/well of CXCR4 Membrane Preparation or Wild-Type Chem1 (Catalog # HTS000MC1) were incubated with 0.13 nM [^{125}I]-SDF-1α and increasing concentrations of unlabeled SDF-1α, and were subjected to filtration binding. A ≥4-fold signal:background ratio was obtained. The data are from a representative lot.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I]-SDF-1α Binding: 6.9 pmol/mg protein
K_d for [^{125}I]-SDF-1α Binding: 0.9 nM
Signal:Background: ≥4-fold

TRANSFECTION: Full-length human CXCR4 cDNA (Accession Number: M99293)

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 non-binding 96-well plate, and incubated for 2 h at room temperature. Prior to filtration, an FC 96-well harvest plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is then 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]-SDF-1α (PerkinElmer # NEX346)

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 4-fold signal:background ratio with [^{125}I]-SDF-1α at 0.13 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris, pH 7.4, 10% glycerol, and 1% BSA.

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. Arakaki R, et al. (1999). T134, a small-molecule CXCR4 inhibitor, has no cross-drug resistance with AMD3100, a CXCR4 antagonist with a different structure. J Virol. 73:1719-23

2. Feng Y, *et al*. (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272:872-7.

3. Kucia M, *et al*. (2004). CXCR4-SDF-1 signaling, locomotion, chemotaxis and adhesion. *J. Mol. Histol*. 35:233-45

4. Muller A*, et al*. (2001). Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410:50-6.

5. Rubin JB, *et al*. (2003). A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc. Natl. Acad. Sci. USA*. 100:13513-8.

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