

PRODUCT DATASHEET
ChemiScreen™ CXCR7 Chemokine Membrane Preparation

CATALOG NUMBER: HTS138M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 2 mg/mL

BACKGROUND: The orphan receptor RDC1 is phylogenetically related to chemokine receptors, and its gene (CMKOR1, chemokine orphan receptor 1) occupies a chromosomal location near the genes for other CXC receptors. Several immune system cells, including T lymphocytes, mature dendritic cells and memory B cells, express RDC1 (Infantino *et al.*, 2006). A subset of HIV and SIV strains use RDC1 as a coreceptor (Shimizu *et al.*, 2000). The chemokine SDF-1/CXCL12, which binds to CXCR4, has been shown also to bind and activate RDC1 to promote chemotaxis of T lymphocytes; thus, CXCR7 has been proposed to be the new name for RDC1 (Balabanian *et al.*, 2005). I-TAC, one of the chemokines thought to bind to CXCR3 only, has been reported to distinguish CXCR7 from CXCR4 in radioligand binding experiments (Burns *et al.* 2006). CXCR7 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 and antagonists at CXCR7. The membrane preparations exhibit a K_d of 0.32 nM for [¹²⁵I]-SDF-1α. With 0.25 nM [¹²⁵I]-SDF-1α, 10 μg/well CXCR7 Membrane Prep typically yields greater than 4-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

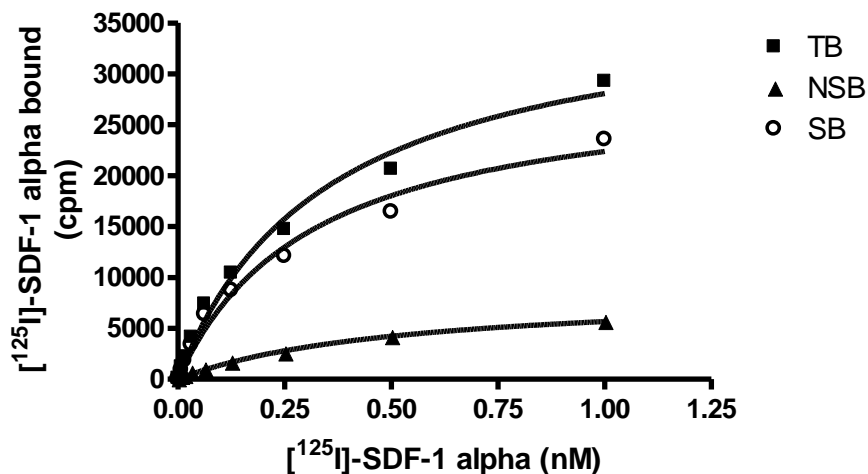


Figure 1. Saturation binding for CXCR7. 10 μg/well CXCR7 Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled SDF-1α in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled SDF-1α. Specific binding (SB) was determined by subtracting NSB from TB. Data obtained from a representative lot.

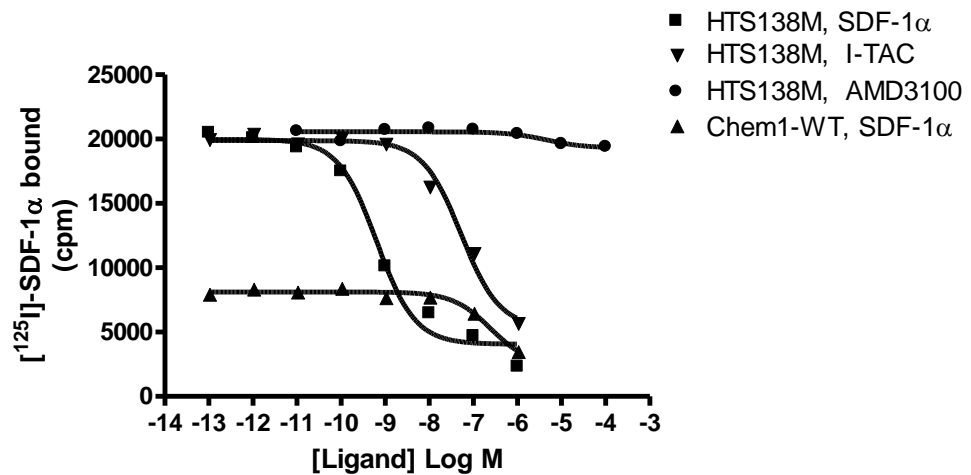


Figure 2. Competition binding for CXCR7. 10 μ g/well CXCR7 Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.25 nM 125 I-labeled SDF-1 α and increasing concentrations of unlabeled SDF-1 α , ITAC, and AMD 3100. More than 4- fold signal:background was obtained with unlabeled SDF-1 α . Data obtained from a representative lot.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with CXCR7 membrane prep and unlabeled SDF-1 α .

	10 μ g/well
Signal:background	4.92
Specific binding (cpm)	15919

SPECIFICATIONS: 1 unit = 10 μ g
 B_{max} for [125 I]- SDF-1 α binding: 0.51 pmol/mg protein
 K_d for [125 I]- SDF-1 α binding: ~0.32 nM

TRANSFECTION: Full-length human CXCR7 cDNA encoding CXCR7 (Accession Number: NM_0203011)

HOST CELLS: Chem-1, an adherent mammalian cell line with minimum amount of endogenous CXCR7 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, 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] SDF-1 α (Perkin Elmer#: NEX-346)

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 4-fold signal:background with ¹²⁵I-labeled SDF-1 α at 0.25 nM

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 2 mg/mL in packaging buffer, and dispensed at 1 mL/vial. Vials were 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. Balabanian K *et al.* (2005) The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. *J. Biol. Chem.* 280: 35760-37566.
 2. Infantino S *et al.* (2006) Expression and regulation of the orphan receptor RDC1 and its putative ligand in human dendritic and B cells. *J. Immunol.* 176: 2197-2207.
 3. Shimizu N *et al.* (2000) A putative G protein-coupled receptor, RDC1, is a novel coreceptor for human and simian immunodeficiency viruses. *J. Virol.* 74: 619-626.
 4. Burns J *et al.* (2006) A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *JEM* 203: 2201-2213.

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