CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN CXCR7 CHEMOKINE RECEPTOR

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). Chemicon's 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 Kd of 0.32 nM for [^{125}I]-SDF-1α. With 0.25 nM [^{125}I]-SDF-1α, 10µg/well CXCR7 Membrane Prep typically yields greater than 4-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

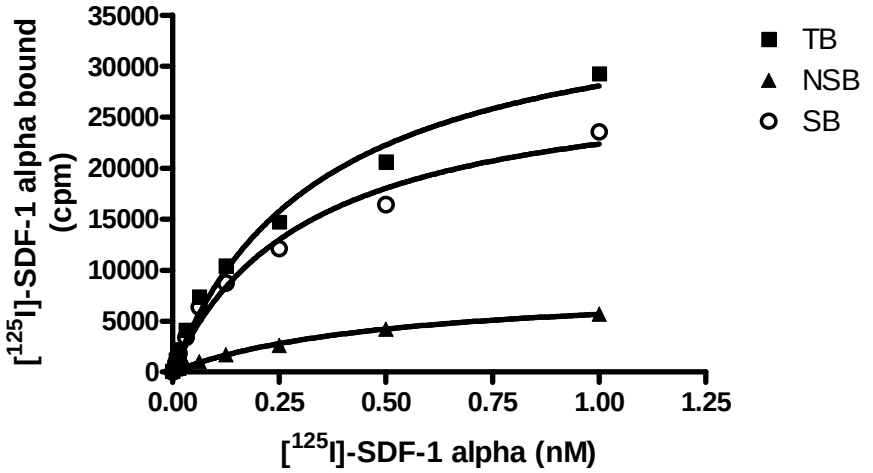

Figure 1. Saturation binding for CXCR7. 10 µg/well CXCR7 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled SDF-1α in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled SDF-1α. Specific binding (SB) was determined by subtracting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

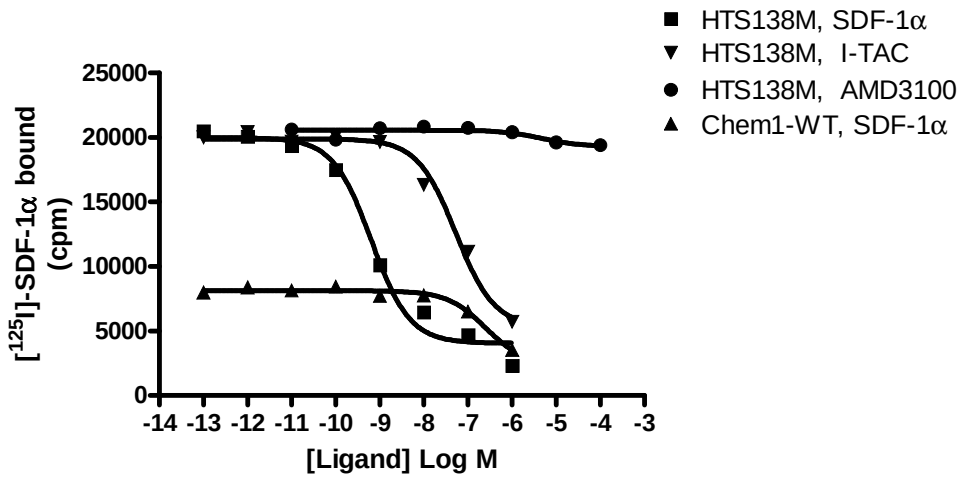

Figure 2. Competition binding for CXCR7. 10 µg/well CXCR7 Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.25 nM ^{125}I-labeled SDF-1α and increasing concentrations of unlabeled SDF-1α, ITAC, and AMD 3100. More than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

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

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{125}I]- SDF-1α binding: 0.51 pmol/mg protein
K_d for [^{125}I]- SDF-1α 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4℃

Radioligand: [^{125}I] SDF-1α (Perkin Elmer#**:** NEX-346)
Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4℃.

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 ^{125}I-labeled SDF-1α 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^{o}C.

STORAGE/HANDLING:

Store at –70℃. Product is stable for at least 6 m onths from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

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.

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.

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.

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

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.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION