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PRODUCT DATASHEET

ChemiScreen™ CXCR6 Chemokine Receptor Membrane Preparation

CATALOG NUMBER:	HTS054M	**QUANTITY:**	200 units
LOT NUMBER:	SC20161103	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: The GPCR CXCR6 (previously known as BONZO, STRL33 and TYMSTR) binds selectively to the free chemokine domain of CXCL16, which is derived from a membrane-bound precursor containing a CXC-containing chemokine domain, a glycosylated mucin-like domain, and a transmembrane domain (Wilbanks *et al.*, 2001) CXCR6 is selectively expressed on Th1, Th2 and Tr1 T cell subsets, whereas CXCL16 is expressed on monocytes/macrophages and dendritic cells (Tabata *et al.*, 2005). CXCR6 promotes migration of activated lymphocytes to sites of inflammation in tissues such as liver and synovium (Nanki *et al.*, 2005, Sato *et al.*, 2005). CXCR6 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 antagonists of CXCR6 interactions with its ligands.

APPLICATIONS: GTPγS Binding and Radioligand binding assay

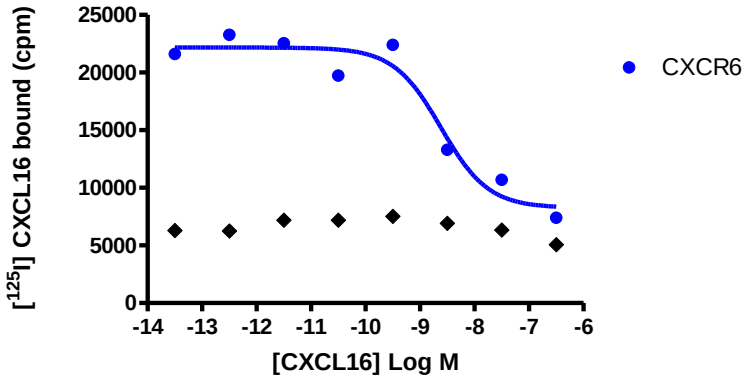

Figure 1. Competition radioligand binding for CXCR6. 10 μg/well CXCR6 Membrane Preparation (HTS054M) and Wild-Type Chem-1 Membrane Preparation (HTS000MC1) was incubated with 0.5 nM ^{125}I-labeled CXCL16 and increasing concentrations of unlabeled human recombinant CXCL16. Representative sample data.

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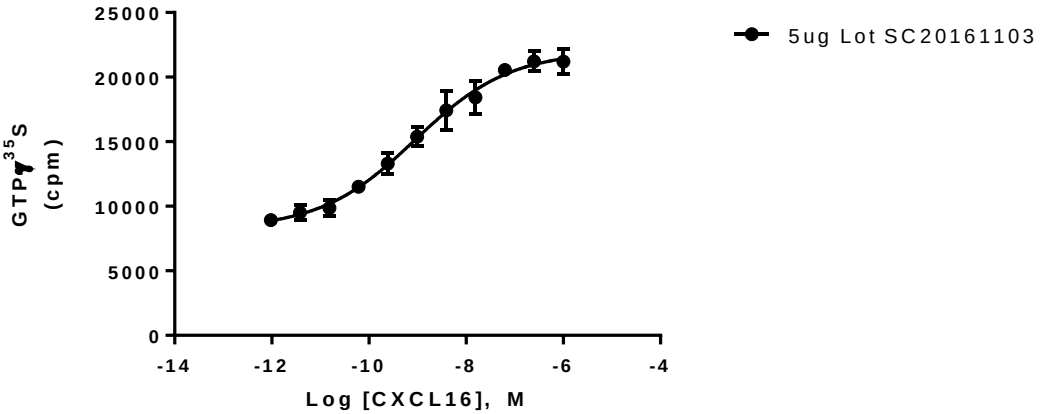

Figure 2. Binding of [^{35}S]-GTPγS to CXCR6 membrane preparation. CXCR6 Membrane Preparation (catalog # HTS054M) at 1 unit per well, were incubated with 0.3 nM [^{35}S]-GTPγS, 0.5 μM GDP and increasing amounts of unlabeled CXCL16. Bound radioactivity was determined by filtration and scintillation counting. Sample data from a representative lot.

SPECIFICATIONS: 1 unit = 5ug for GTPγS binding assay

TRANSFECTION: Human CXCR6 (Accession number AY322543)

Species: Human

HOST CELLS: Chem-5, an adherent cell line derived from Chem-1 cells

RECOMMENDED RADIOLIGAND BINDING 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, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) 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] CXCL16 (Perkin Elmer catalog # NEX398)

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

RECOMMENDED [^{35}S]-GTPγS BINDING ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 μM GDP in a nonbinding 96-well plate. Unlabeled agonist was added to the final concentration and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (EMD Millipore cat # MAHF B1H) previously pre-wetted with water. The plate is washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4, then dried and counted.

One package contains enough membranes for at least 200 assays (units)

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml 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. Do not freeze and thaw.

REFERENCES:

1. Nanki T *et al*. (2005) Pathogenic role of the CXCL16-CXCR6 pathway in rheumatoid arthritis. *Arthritis Rheum*. 52(10):3004-14.
2. Sato T *et al*. (2005) Role for CXCR6 in recruitment of activated CD8+ lymphocytes to inflamed liver. *J. Immunol.* 174: 277-83.
3. Tabata S *et al.* (2005) Distribution and kinetics of SR-PSOX/CXCL16 and CXCR6 expression on human dendritic cell subsets and CD4+ T cells. *J. Leukoc. Biol.* 77: 777-86.
4. Wilbanks A *et al.* (2001) Expression cloning of the STRL33/BONZO/TYMSTR ligand reveals elements of CC, CXC, and CX3C chemokines. *J. Immunol.* 166: 5145-5154.