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PRODUCT DATASHEET

ChemiScreen™ CCR6 Chemokine Membrane Preparation

CATALOG NUMBER:	HTS011M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND:

CCR6 is a GPCR that binds to a single chemokine, CCL20 (also known as MIP-3α, LARC or Exodus-1), as well as several members of the beta-defensin family of antimicrobial peptides (Schutyser, 2003). Immature dendritic cells, B-cells, and effector/memory T-cells express CCR6, and the receptor promotes inflammation during the development of rheumatoid arthritis, inflammatory bowel disease, and graft-versus-host disease (Varona et al., 2003; Kleinewietfeld et al., 2005; Varona et al., 2005). CCR6 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 CCR6 interactions with MIP-3α. The membrane preparations exhibit a Kd of 0.09 nM for [^{125}I]-MIP-3α. With 0.3 nM [^{125}I]-MIP-3α, 5 μg/well and 10 μg/well CCR6 Membrane Prep typically yield greater than 6-fold and 8-fold signal-to-background ratios, respectively.

APPLICATIONS:

Radioligand binding assay

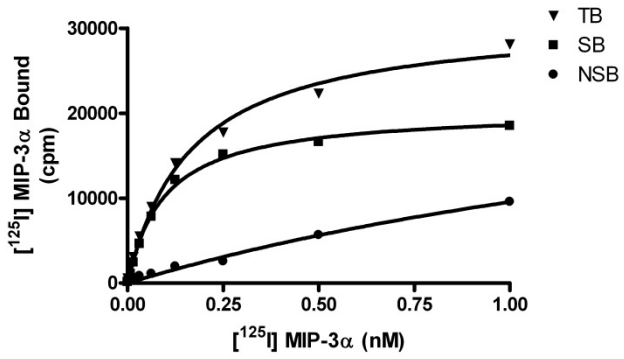

Figure 1. Saturation binding for CCR6. 5 μg/well CCR6 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled MIP-3α in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled MIP-3α. Specific binding (SB) was determined by subtracting NSB from TB.

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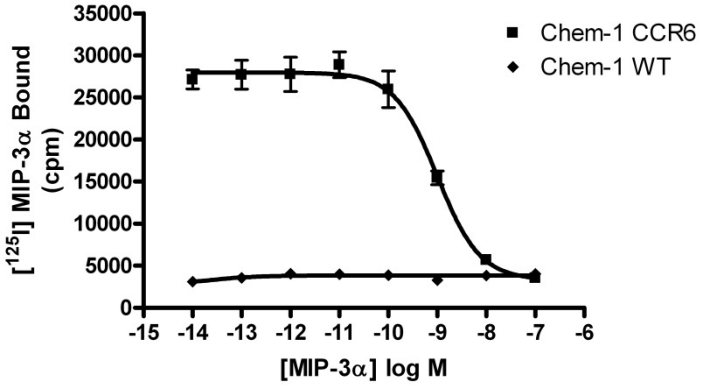

Figure 2. Competition binding for CCR6. 10 µg/well CCR6 Membrane Preparation and Wild-Type Chem-1 Membrane Preparation (catalog #HTS000MC1) were incubated with 0.3 nM ^{125}I-labeled MIP-3α and increasing concentrations of unlabeled MIP-3α, and more than 5- fold signal: background was obtained.

SPECIFICATIONS: B_{max}: 2.7 pmol/mg; Kd: 0.09 nM

Species: Human CCR6 (Accession number U45984)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CCR6 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°C.

Radioligand: [^{125}I] MIP-3α. (Perkin Elmer# NEX371)

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 ^{125}I-labeled MIP-3α at 0.3 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. Kleinewietfeld M et al. (2005) CCR6 expression defines regulatory effector/memory-like cells within the CD25(+)CD4+ T-cell subset. *Blood* 105: 2877-86.
2. Schutyser E (2003) The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev.* 14: 409-26.
3. Varona R et al. (2003) CCR6 has a non-redundant role in the development of inflammatory bowel disease. *Eur. J. Immunol.* 33: 2937-46.
4. Varona R et al. (2005) CCR6 regulates CD4+ T-cell-mediated acute graft-versus-host disease responses. *Blood* 106: 18-26.