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PRODUCT DATASHEET

ChemiScreen™ CCR2B CHEMOKINE Membrane Preparation

CATALOG NUMBER:	HTS007M	**QUANTITY:**	200 units
LOT NUMBER:	SC601752	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

CCR2 is a GPCR that is expressed on monocytes, dendritic cells, natural killer cells, basophils and neutrophils, and binds to MCP-1, 3 and 4, members of the MCP (monocyte chemoattractant protein) family of chemokines (Olson and Ley, 2002). Alternative splicing of the CCR2 gene results in two variants, CCR2A and CCR2B, which differ in their C-terminal tails (Wong and Charo, 1997). CCR2 plays a protective role in some conditions: mice lacking the gene encoding CCR2 develop age-related macular degeneration (Ambati *et al.*, 2003), and in experimental models of rheumatoid arthritis, CCR2-null mice develop a phenotype of greater severity than wild-type mice (Quinones *et al.*, 2004). However, CCR2 appears to promote the pathogenesis of graft-versus-host disease and pulmonary and kidney fibrosis (Kitagawa *et al.*, 2004; Rao *et al.*, 2003). CCR2B 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 CCR2B interactions with MCP-1.

APPLICATIONS:

Radioligand binding assay

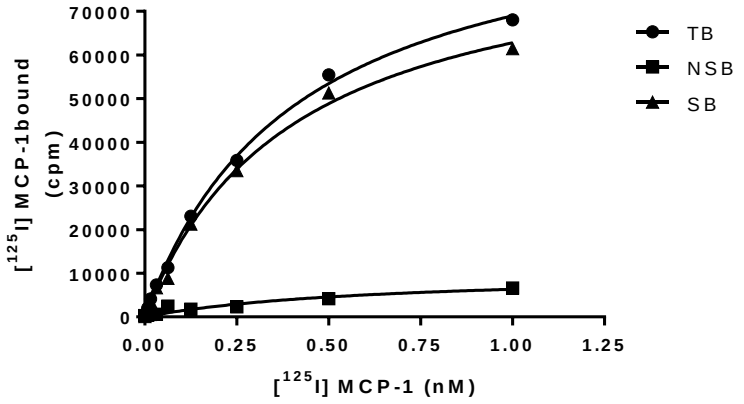

Figure 1. Saturation binding for CCR2B. 5 µg/well CCR2B Membrane Preparation was incubated with increasing amount of ^{125}I-labeled MCP-1 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled MCP-1. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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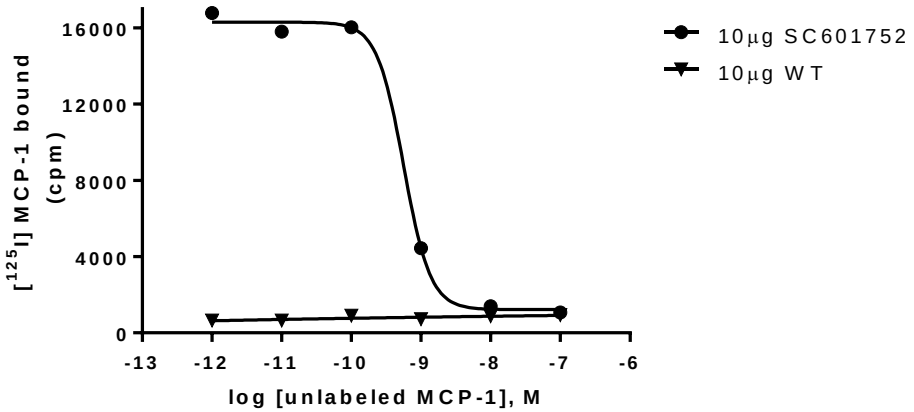

Figure 2. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of CCR2B membrane prep. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 3.16 pmol/mg
K_d: 0.4 nM
Signal:background: ≥5-fold

Species: Human CCR2B (Accession number U03905)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CCR2B 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] MCP-1 (Perkin Elmer# NEX332)

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 5-fold signal: background with ^{125}I-labeled MCP-1 at 0.1 nM.

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. Ambati J *et al.* (2003) An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat. Med.* 9: 1390-1397.
2. Kitagawa K *et al.* (2004) Blockade of CCR2 ameliorates progressive fibrosis in kidney. *Am. J. Pathol.* 165: 237-246.
3. Olson TS and Ley K (2002) Chemokines and chemokine receptors in leukocyte trafficking. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283: R7-28.
4. Quinones MP *et al.* (2004) Experimental arthritis in CC chemokine receptor 2-null mice closely mimics severe human rheumatoid arthritis. *J. Clin. Invest*. 113: 856-66.
5. Rao AR *et al.* (2003) CC chemokine receptor 2 expression in donor cells serves an essential role in graft-versus-host-disease. *J. Immunol.* 171: 4875-4885.
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