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CHEMISCREEN™ MEMBRANE PREPARATION
RHESUS MACAQUE RECOMBINANT CCR5 CHEMOKINE RECEPTOR

CATALOG NUMBER:	HTS010M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION PER VIAL:**	1 mL, 1 mg/mL

BACKGROUND:

CCR5 is the receptor for CC chemokines MIP-1α, MIP-1β, and RANTES (Raport *et al*., 1996), and is preferentially expressed on Th1 lymphocytes (Loetscher *et al*., 1998). CCR5 is a coreceptor for macrophage-tropic HIV, and its ligands potently inhibit HIV replication in human leukocytes (Cocchi *et al*., 1995). In addition, HIV-infected patients with the nonfunctional CCR5Δ32 allele exhibit delayed onset of AIDS symptoms (Samson *et al.*, 1996), and pharmacological antagonism of CCR5 inhibits HIV-1 infection (Strizki *et al*., 2001). Preclinical testing of small molecule antagonists of CCR5 has been hampered by low affinity of the compounds to rodent and dog CCR5, but two such compounds, maraviroc and AD101, have been shown to have potent antagonist activity at rhesus macaque CCR5, which differs from human CCR5 by 8 amino acids (Napier *et al*., 2005; Billick *et al*., 2004). One antagonist of human CCR5, SCH-C, does not block HIV entry through rhesus macaque CCR5, and one amino acid difference is responsible for the functional difference (Billick *et al*., 2004). Millipore's rhesus macaque CCR5 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 of rCCR5. The membrane preparations exhibit a Kd of 0.32 nM for [^{125}I]-MIP-1α. With 0.25 nM [^{125}I]-MIP-1α, 5 μg/well rCCR5 Membrane Prep typically yields greater than 7-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay and GTPγS binding.

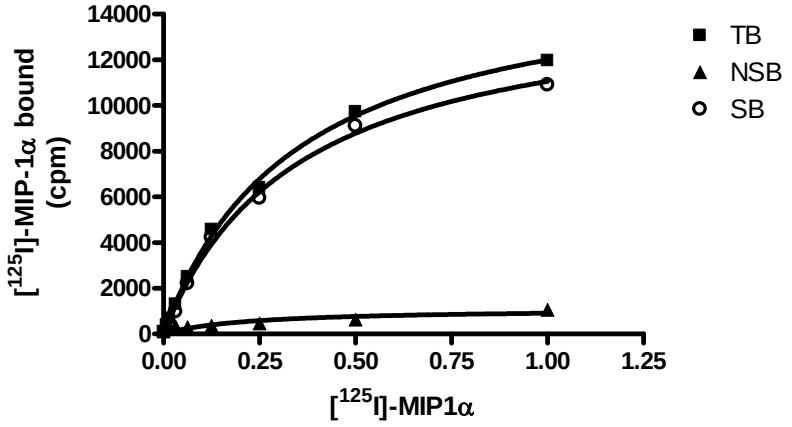

Figure 1. Saturation binding for rhesus macaque CCR5. 5 μg/well CCR5 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled MIP-1α in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled MIP-1α. Specific binding (SB) was determined by subtracting NSB from TB.

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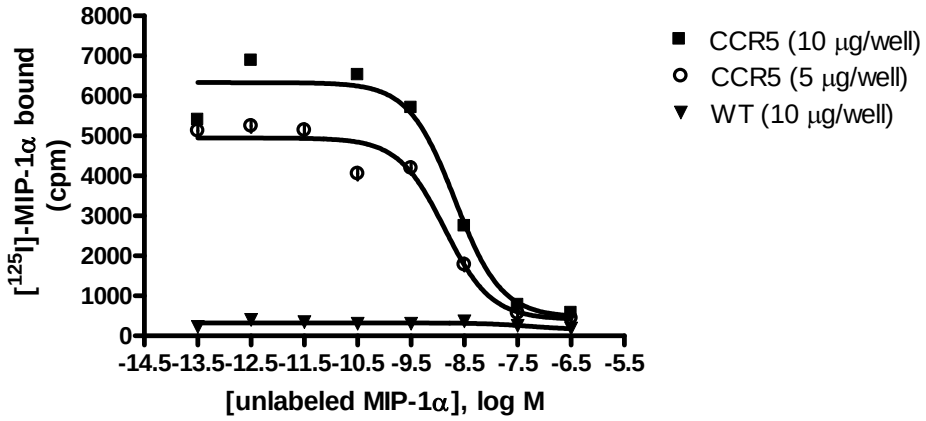

Figure 2. Competition binding for rhesus macaque CCR5. CCR5 Membrane Preparation (5 or 10 μg/well) and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.15 nM ^{125}I-labeled MIP-1α and increasing concentrations of unlabeled MIP-1α. More than 7-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with CCR5 Receptor membrane prep.

	10 μg/well	5 μg/well
Signal:background	13.6	12.1
Specific binding (cpm)	5868.9	4538

SPECIFICATIONS: 1 unit = 5 μg
B_{max} for [^{125}I]-MIP-1α binding: 1.9 pmol/mg protein
K_d for [^{125}I]-MIP-1α binding: ~0.35 nM

TRANSFECTION: Full-length rhesus macaque CCR5 cDNA encoding CCR5 (Accession Number: NM_001042773.2)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

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, an FC 96-well harvest plate (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]-MIP-1α (Perkin Elmer#**:** NEX-298)

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 7-fold signal:background with ^{125}I labeled MIP-1α at 0.15 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 1 mg/ml in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Maintain frozen at –70°C for up to 2 years. Do not freeze and thaw.

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