MILLIPORE

CHEMICON
now part of Millipore

CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN CCR7 CHEMOKINE RECEPTOR

CATALOG NUMBER:	HTS012M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	4 mL, 0.5 mg/mL

BACKGROUND:

CCR7 is a GPCR that binds to CC chemokine ligands MIP-3β (ELC/CCL19) and 6Ckine (CCL21) (Yoshida *et al*., 1997). These ligands are expressed in the secondary lymphoid organs, and binding to CCR7 expressed in naïve T cells, B cells and dendritic cells directs migration of these cells to sites of antigen presentation (Förster *et al*., 1999). Inhibition of CCR7/ligand interactions inhibits contact sensitivity, delayed type hypersensitivity, and graft vs host disease in experimental models (Förster *et al*., 1999; Sasaki *et al*., 2003). In addition, CCR7 expression by breast cancer, melanoma and other malignant cells are associated with lymph node metastasis (Müller *et al*., 2001; Payne and Cornelius, 2002). Chemicon's CCR7 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 CCR7 interactions with 6Ckine and MIP-3β. The membrane preparations exhibit a Kd of 0.2 nM for [^{125}I]- MIP-3β. With 10 μg/well CCR7 Membrane Prep and 0.1 nM [^{125}I]- MIP-3β, a greater than 7-fold signal-to-background ratio is obtained.

APPLICATIONS:

Radioligand binding assay, and GTPγS binding.

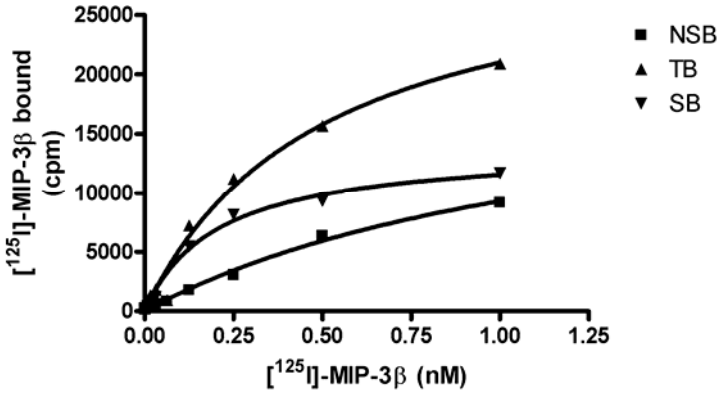

Figure 1. Saturation binding for CCR7. 10 μg/well CCR7 Membrane Preparation was incubated with increasing amount of [^{125}I]- MIP-3β in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled MIP-3β. Specific binding (SB) was determined by subtracting NSB from TB.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered worldwide
www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com

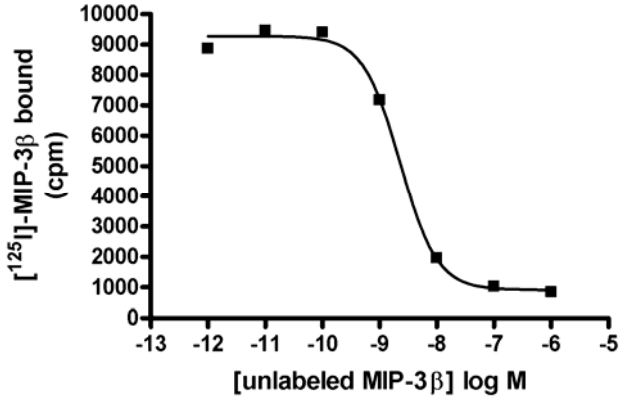

Figure 2. Competition binding for CCR7. CCR7 Membrane Preparation (10 μg/well) was incubated with 0.1 nM [^{125}I]-MIP-3β and increasing concentrations of unlabeled MIP-3β, and more than 7- fold signal:background was obtained.

	10 μg/well
Signal:background	10.2
Specific binding	8362

Table 1. Signal:background and specific binding values obtained in a competition binding assay with CCR7 membrane preparation.

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
Bmax: 3.3 pmol/mg
K_d: 0.2 nM

Species: Human CCR7 (Accession number L08176)

HOST CELLS: Chem-2, a suspension mammalian cell line without any endogenous CCR7 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.

MILLIPORE

CHEMICON

now part of Millipore

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-3β (Perkin Elmer #NEX370)

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 an unit is the amount of membrane that will yield greater than 7-fold signal:background with ^{125}I-labeled MIP-3β at 0.1 nM

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein were adjusted to the indicated concentration in 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.

REFERENCES:

Yoshida R *et al.* (1997) Molecular cloning of a novel human CC chemokine EBI1-ligand chemokine that is a specific functional ligand for EBI1, CCR7. *J. Biol. Chem.* 272: 13803-9.

Förster R *et al*. (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99: 23-33.

Sasaki M *et al.* (2003) Antagonist of secondary lymphoid-tissue chemokine (CCR ligand 21) prevents the development of chronic graft-versus-host disease in mice. *J. Immunol.* 170: 588-96.

Müller A *et al.* (2001) Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410: 50-56.

Payne AS and Cornelius LA (2002) The role of chemokines in melanoma tumor growth and metastasis. *J. Invest. Dermatol.* 118: 915-922.

For research use only; not for use as a diagnostic.

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.*

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered worldwide
www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com