

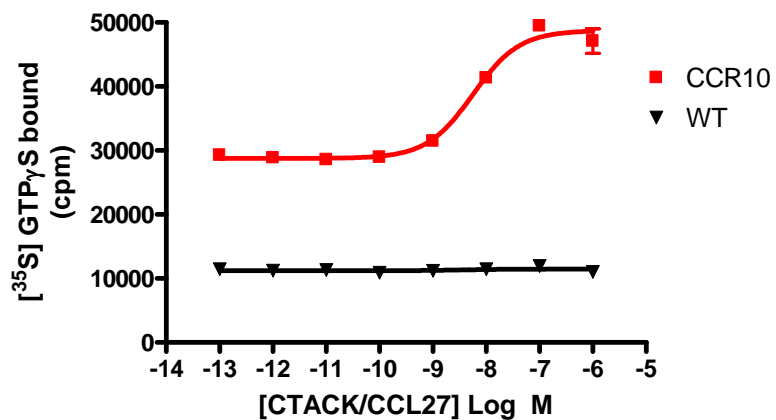


# CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN CCR10 CHEMOKINE RECEPTOR

**CATALOG NUMBER:** HTS014M      **QUANTITY:** 200 units  
**LOT NUMBER:**      **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

**BACKGROUND:** CCR10, previously known as GPR2, is a GPCR that is expressed in dermal endothelial cells, fibroblasts, melanocytes, and circulating T cells and PBMCs (Homey *et al.*, 2000). Two chemokines, CTACK/CCL27 and MEC/CCL28, bind to CCR10 and stimulate migration and calcium flux (Pan *et al.*, 2000; Wang *et al.*, 2000). Expression of CTACK by keratinocytes, and CCR10 by lymphocytes from skin of patients with psoriasis and dermatitis, implicates this receptor ligand pair in skin inflammation. In addition, disruption of CCL27-CCR10 interaction reduces skin inflammation induced by allergen (Homey *et al.*, 2002). CCR10 expressed on IgA antibody-secreting cells also mediates their migration to CCL28 expressed in the lactating mammary gland, and subsequent IgA accumulation in milk (Wilson and Butcher, 2004). Millipore's CCR10 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 CCR10 interactions with its ligands. The membrane preparations exhibit an EC<sub>50</sub> of 47 nM for CTACK/CCL27 in a GTP $\gamma$ S binding assay.

**APPLICATIONS:** GTP $\gamma$ S Binding Assay.



**Figure 1. Binding of [<sup>35</sup>S]-GTP $\gamma$ S to CCR10 membrane preparation.** 5  $\mu$ g/well CCR10 Membrane Preparation (catalog # HTS014M) and Chem-1 Wild-Type Membrane Preparation (WT; Millipore catalog # HTS000MC1) were incubated with 0.3 nM [<sup>35</sup>S]-GTP $\gamma$ S and increasing amounts of unlabeled CTACK/CCL27. Bound radioactivity was determined by filtration and scintillation counting.



## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN CCR10 CHEMOKINE RECEPTOR

**SPECIFICATIONS:** EC<sub>50</sub> for CTACK/CCL27: ~5.6 nM in a GTP $\gamma$ S binding assay

**Species:** Full length human CCR10 (Accession number AF215981) with a proprietary mutation in the cytoplasmic domain to enhance cell surface expression.

**HOST CELLS:** Chem-1, an adherent mammalian cell line with no detectable CCR10 expression.

**GTP $\gamma$ S ASSAY CONDITIONS:** Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [<sup>35</sup>S]-GTP $\gamma$ S (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl<sub>2</sub>/0.5  $\mu$ M GDP in a nonbinding 96-well plate. Unlabeled ligand was added to the final concentration indicated in Figure 1 (final volume 100  $\mu$ L), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (Millipore MAHF B1H) previously prewetted 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 vial contains enough membranes for at least 200 assays (units), where one unit is the amount of membrane that will yield greater than 1000 cpm specific CTACK/CCL27-stimulated [<sup>35</sup>S]-GTP $\gamma$ S binding.

**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 the indicated concentration in 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:**

Homey B., *et al.* (2000) The orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) bind the skin-associated chemokine CCL27 (CTACK/ALP/ILC). *J. Immunol.* 164: 3465-3470.

Homey B., *et al.* (2002) CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. *Nat. Med.* 8: 157-165.

Pan J., *et al.* (2000) A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. *J. Immunol.* 165: 2943-2949.

Wang W., *et al.* (2000) Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). *J. Biol. Chem.* 275: 22313-22323.

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