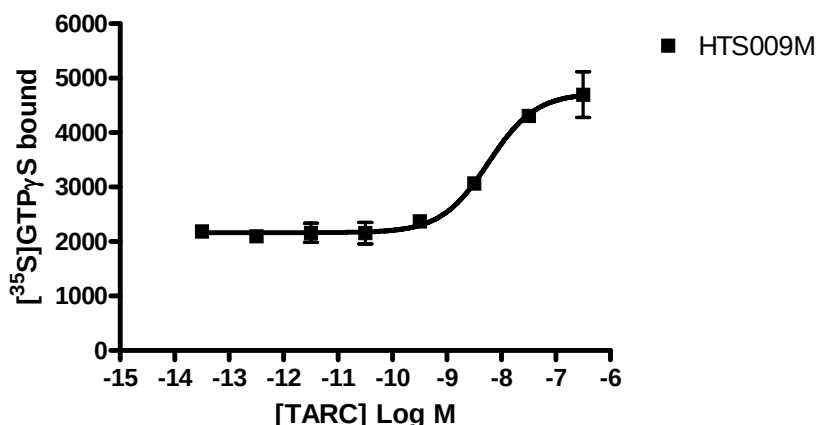


## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN CCR4 CHEMOKINE RECEPTOR

|                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                       |               |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|---------------|
| <b>CATALOG NUMBER:</b> | HTS009M                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <b>QUANTITY:</b>                      | 200 units     |
| <b>LOT NUMBER:</b>     | R0705E0024                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | <b>VOLUME/CONCENTRATION PER VIAL:</b> | 1 mL, 1 mg/mL |
| <b>BACKGROUND:</b>     | CCR4 is a GPCR that is activated by chemokines TARC (thymus and activation regulated chemokine) and MDC (macrophage-derived chemokine) (Olsen and Ley, 2001). NK cells, Th2 cells, cutaneous memory T cells, macrophages and platelets express CCR4 and respond to TARC and MDC (Andrew <i>et al.</i> , 2001; Inngjerdingen <i>et al.</i> , 2000; Gear <i>et al.</i> , 2001; Soler <i>et al.</i> , 2003). CCR4-null mice display reduced airway hyperresponsiveness to fungal spores, lipopolysaccharide-induced endotoxic shock, and cardiac allograft rejection (Chvatchko <i>et al.</i> , 2000; Schuh <i>et al.</i> , 2002; Huser <i>et al.</i> , 2005). In addition, antibody – mediated antagonism of MDC inhibits development of insulinitis and diabetes in NOD mice (Kim <i>et al.</i> , 2002). Chemicon's CCR4 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 at CCR4. The cell line exhibits a calcium response with EC50 of 3.31nM for TARC. The membrane preparations exhibit EC50 of 5.76 nM for TARC in a GTP $\gamma$ S binding assay. |                                       |               |
| <b>APPLICATIONS:</b>   | Radioligand binding assay and GTP $\gamma$ S binding.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                       |               |



**Figure 1. Binding of [<sup>35</sup>S]-GTP $\gamma$ S to CCR<sub>4</sub> membrane preparation.** 5  $\mu$ g/well CCR4 Membrane Preparation (catalog # HTS009M) was incubated with 0.1 nM [<sup>35</sup>S]-GTP $\gamma$ S and increasing amounts of unlabeled TARC. Bound radioactivity was determined by filtration and scintillation counting.

**SPECIFICATIONS:** 1 unit = 5  $\mu$ g  
EC50 in GTP $\gamma$ S binding assay by TARC: ~ 5.76 nM

**TRANSFECTION:** Full-length human CCR4 cDNA encoding CCR4 (Accession Number: X85740)

**HOST CELLS:** Chem-1, an adherent mammalian cell line with minimum amount of

endogenous CCR4 expression.

**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 0.1 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 TARC is 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 a GF/B filter plate (Millipore MAHF B1H) previously prewetted with water, and washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4. The plate is 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 to TARC-stimulated [<sup>35</sup>S]-GTP $\gamma$ S binding.

The CCR4 membrane preparation is expected to be functional in a radioligand binding assay; however, the end user will need to determine the optimal radiolabeled ligand for use with this product.

**PRESENTATION:**

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane protein was adjusted to 1 mg/ml 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:**

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