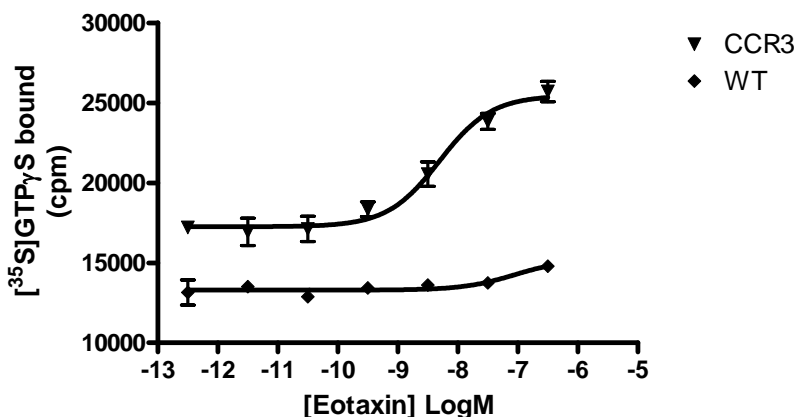


**CHEMISCREEN™ MEMBRANE PREPARATION  
RECOMBINANT HUMAN CCR3 CHEMOKINE RECEPTOR**

**CATALOG NUMBER:** HTS008M      **QUANTITY:** 200 units  
**LOT NUMBER:**      **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

**BACKGROUND:** Eosinophils are major effector cells implicated in a number of chronic inflammatory diseases in humans, particularly bronchial asthma and allergic rhinitis. The chemokine receptor 3 (CCR3), a GPCR activated by chemokines eotaxin 1/2, MCP-3, MCP-4, and RANTES, mediates selective recruitment of eosinophils into tissue and thus has recently become an attractive biological target for therapeutic intervention (Fujisawa *et al.*, 2000). It is widely expressed on cells involved in allergic inflammation, such as basophils, mast cells, airway epithelial cells, and potentially TH<sub>2</sub> T-lymphocytes. Allergen-induced eosinophil infiltration into airways is reduced or eliminated in CCR3 and eotaxin 1/2 knockout mice and in mice treated with antibodies directed against CCR3 (Grimaldi *et al.*, 1999; Fulkerson *et al.*, 2006). CCR3 antagonists are currently being developed for the treatment of asthma and other allergic disorders. Millipore's CCR3 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 CCR3 interactions with its ligands. The cell line exhibits a calcium response with EC<sub>50</sub>s of 6.5 nM for eotaxin. The membrane preparations exhibit EC<sub>50</sub>s of 4.9 nM for eotaxin in a GTP $\gamma$ S binding assay.

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



**Figure 1. Binding of [<sup>35</sup>S]-GTP $\gamma$ S to CCR3 membrane preparation.** 5  $\mu$ g/well CCR3 Membrane Preparation (catalog # HTS008M) was incubated with 0.3 nM [<sup>35</sup>S]-GTP $\gamma$ S and increasing amounts of unlabeled eotaxin. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: 1 unit = 5 µg  
EC50 in GTP $\gamma$ S binding assay by Eotaxin: ~ 4.9 nM

Species: human CCR3 cDNA encoding CCR3 (Accession Number: U28694)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G $\alpha$ 15.

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 µM GDP in a nonbinding 96-well plate. Unlabeled eotaxin was added to the final concentration indicated in Figure 1 (final volume 100 µL), 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. 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 eotaxin-stimulated [<sup>35</sup>S]-GTP $\gamma$ S binding.

The CCR3 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:**

Fujisawa T *et al.* (2000). Chemokines induce eosinophil degranulation through CCR-3. *J. Allergy Clin. Immunol.* 106: 507–513.

Fulkerson PC *et al.* (2006) A central regulatory role for eosinophils and the eotaxin/CCR3 axis in chronic experimental allergic airway inflammation. *Proc. Natl. Acad. Sci. USA* 103: 16418-16423.

Grimaldi JC *et al.* (1999). Depletion of eosinophils in mice through the use of antibodies specific for C-C chemokine receptor 3 (CCR3). *J. Leukoc. Biol.* 65: 846–853.

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