

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 TH₂ 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 EC₅₀s of 6.5 nM for eotaxin. The membrane preparations exhibit EC₅₀s of 8 nM for eotaxin in a GTP γ S binding assay.

APPLICATIONS: GTP γ S Binding Assay.

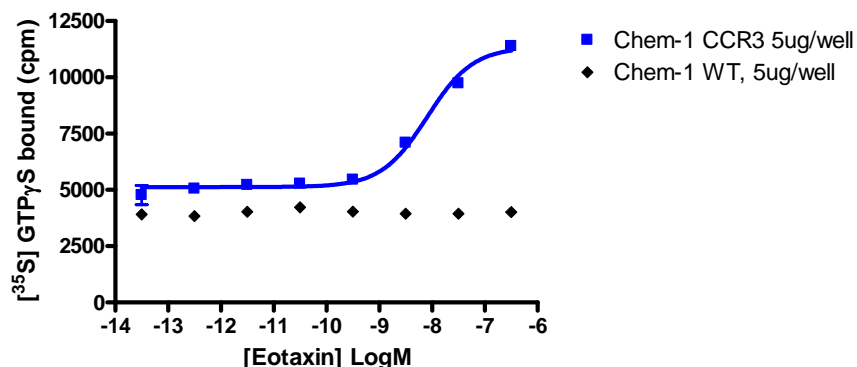


Figure 1. Binding of [³⁵S]-GTP γ S to CCR3 membrane preparation. 5 μ g/well CCR3 Membrane Preparation (catalog # HTS008M) was incubated with 0.3 nM [³⁵S]-GTP γ S and increasing amounts of unlabeled eotaxin. Bound radioactivity was determined by filtration and scintillation counting. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
EC50 in GTP γ S binding assay by Eotaxin: 8 nM
Signal window: >5,000 cpm

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

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

ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [³⁵S]-GTP γ S (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl₂/0.5 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 [³⁵S]-GTP γ 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:

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:

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.



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 μ L 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.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC
PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

©2007 - 2012: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.