eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ CCR8 Chemokine Membrane Preparation

CATALOG NUMBER:	HTS013M	**QUANTITY:**	200 units
LOT NUMBER:	SC113599	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: CCR8 is a GPCR that binds primarily to the chemokine I-309 (CCL1) (Roos *et al*., 1997). CCR8 is expressed primarily on Th2 cells, although its functional role in T cell recruitment to sites of allergic inflammation is controversial (Chensue *et al*., 2001; Goya *et al*., 2003). CCR8+ T-cells are abundant in skin and rare or absent in the GI tract and peripheral blood, indicating a role in skin-homing of T-cells (Schaerli *et al*., 2004). In addition, monocyte-derived dendritic cells express CCR8 and utilize CCR8 during mobilization from skin to the lymph node (Qu *et al*., 2004). CCR8 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 CCR8 interactions with I-309.

APPLICATION: GTPγS Binding Assay

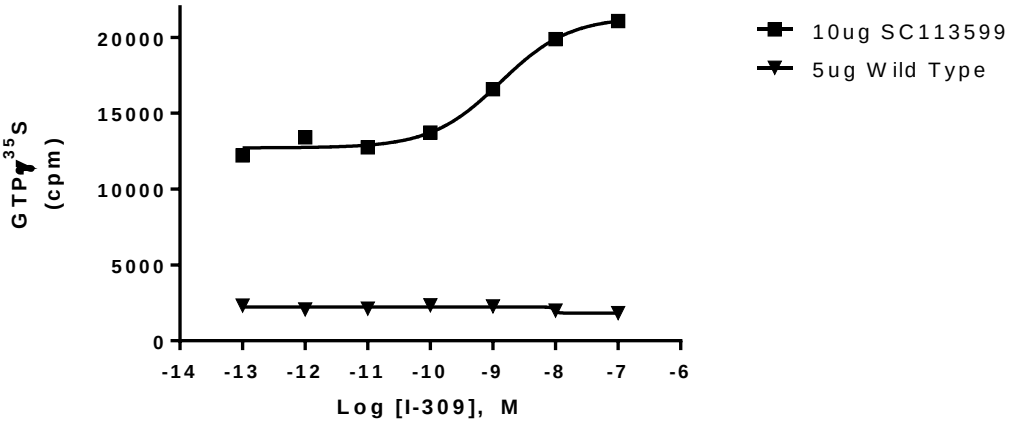

Figure 1. Binding of [^{35}S]-GTPγS to CCR8 Membrane Preparation. 10 µg/well of CCR8 Membrane Preparation (catalog # HTS013M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled I-309. Bound radioactivity was determined by filtration and scintillation counting. Sample data from a representative lot SC113599.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

Template version: 7. Modified Date: 16JAN15. Modified by: LRC

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
EC50 in GTPγS binding assay by I-309: ~1.3nM
Signal window: >2500 cpm

Species: Human CCR8 (Accession number U45983)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CCR8 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in assay buffer in a non-binding 96-well plate. Unlabeled I-309 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 an FB filter plate (EMD Millipore MAHF B1H) previously pre-wetted with water, and washed 3 times (1 mL per well per wash) with cold wash buffer. The plate is dried and counted.

Binding Buffer: 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/10 µM GDP

Radioligand: [^{35}S]-GTPγS (PerkinElmer;# NEG030H)

Wash Buffer: 10 mM Sodium phosphate, pH 7.4.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 1000 cpm specific I-309-stimulated [^{35}S]-GTPγS binding.

Special Note: The CCR8 receptor membrane preparation is expected to be functional in a radioligand binding assay with [^{125}I]-I-309.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris, pH 7.4, 10% glycerol,l and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80^{o}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:

1. Chensue SW *et al*. (2001). Aberrant in vivo T helper type 2 cell response and impaired eosinophil recruitment in CC chemokine receptor 8 knockout mice. J. Exp. Med. 193:573-84.

2. Goya I *et al*. (2003). Absence of CCR8 does not impair the response to ovalbumin-induced allergic airway disease. J. Immunol. 170:2138-46.

3. Qu C *et al*. (2004.) Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. J. Exp. Med. 200:1231-1241.

4. Roos RS *et al*. (1997). Identification of CCR8, the receptor for the human CC chemokine I-309. J. Biol. Chem. 272:17251-17254.

5. Schaerli P *et al*. (2004). A skin-selective homing mechanism for human immune surveillance T cells. J. Exp. Med. 199:1265-75.

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.

Eurofins Pharma Bioanalytics Services US Inc. is an independent member of Eurofins Discovery Services.