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CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN ChemR23 CHEMOATTRACTANT RECEPTOR

CATALOG NUMBER:	HTS071M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

ChemR23 was discovered as an orphan receptor related to the chemoattractant receptors C3a, C5a and FPR1, and expressed on dendritic cells and macrophages (Samson *et al.*, 1998). A ligand for ChemR23 was characterized as chemerin, a 15 kD proteolytically processed protein found in inflammatory sites; a 9 amino acid peptide from the C-terminus of chemerin is sufficient to activate ChemR23 (Wittamer *et al.*, 2003, 2004). Chemerin expressed in lymphoid and microvascular endothelium mediates migration of ChemR23-expressing dendritic cells to lymphoid organs and vasculature at sites of inflammation (Vermi *et al.*, 2005). In addition, a bioactive lipid, resolvin E1, was found to functionally interact with ChemR23 to reduce inflammation (Arita *et al*., 2005). Millipore's ChemR23 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 ChemR23 interactions with its ligands. The membrane preparations exhibit EC50s of 0.37 nM for chemerin in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay.

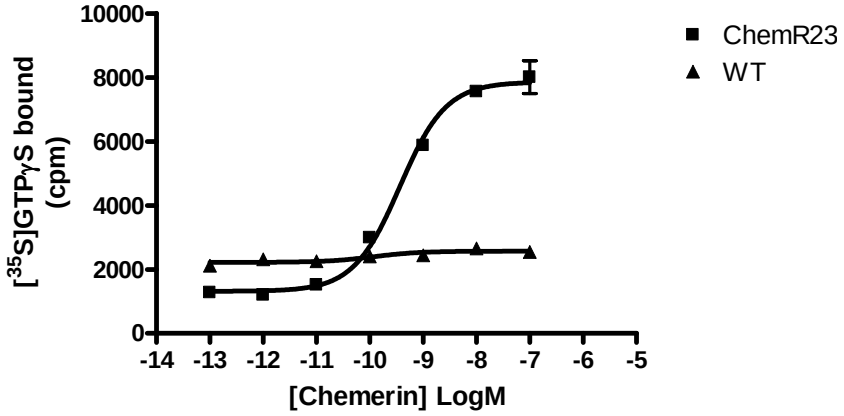

Figure 1. Binding of [^{35}S]-GTPγS to ChemR23 membrane preparation. 5 μg/well ChemR23 Membrane Preparation (catalog # HTS071M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled recombinant human chemerin. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: 1 unit = 5 μg

EC50 in GTPγS binding assay by Chemerin: ~ 0.37 nM

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Species: Full-length human ChemR23 (Accession Number: NM_004072)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.
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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 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 μM GDP in a nonbinding 96-well plate. Unlabeled chemerin 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 chemerin-stimulated [^{35}S]-GTPγS binding.

The ChemR23 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^{o}C.

STORAGE/HANDLING:

Maintain frozen at –70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Arita M *et al.* (2005) Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J. Exp. Med.* 201: 713-722.

Samson M *et al.* (1998) ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. *Eur. J. Immunol.* 28: 1689-1700.

Vermi W *et al.* (2005) Role of ChemR23 in directing the migration of myeloid and plasmacytoid dendritic cells to lymphoid organs and inflamed skin. *J. Exp. Med.* 201:509-515.

Wittamer *et al.* (2003) Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. *J. Exp. Med.* 198: 977-985.

Wittamer *et al.* (2004) The C-terminal nonapeptide of mature chemerin activates the chemerin receptor with low nanomolar potency. *J. Biol. Chem.* 279: 9956-9962.

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