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CHEMISCREEN™ MEMBRANE PREPARATION
HUMAN RECOMBINANT $S1P_2$ LYSOPHOSPHOLIPID RECEPTOR

CATALOG NUMBER:	HTS078M	**QUANTITY:**	200 units
LOT NUMBER:	2022543	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

Sphingosine 1-phosphate (S1P) is a bioactive lipid that binds to and activates a family of GPCRs, $S1P_{1-5}$ (also known as EDG receptors). Interactions between S1P and its receptors mediate cytoskeletal rearrangement and cell migration, with functional consequences in angiogenesis, lymphocyte trafficking, and smooth muscle development (Anliker and Chun, 2004). $S1P_1$ (Edg-1) signals exclusively through G_i, whereas $S1P_2$ (Edg-5) and $S1P_3$ (Edg-3) activate G_i, G_q and $G_{12/13}$ (Windh *et al.*, 1999). Although $S1P_1$ and $S1P_3$ promote cell migration, $S1P_2$ inhibits cell migration in several cell types; these opposing functions appear to result from differences in the ability of each receptor to activate G_i (Arikawa *et al.*, 2003; Sugimoto *et al.*, 2003; Goparaju *et al.*, 2005). Studies with knockout mice indicate that $S1P_2$ and $S1P_3$ have redundant functions in maintaining vascular integrity during embryonic development (Kono *et al.*, 2004). Chemicon's $S1P_2$ 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 $S1P_2$ interactions with its ligands. The cell line exhibits EC50 of 7.2nM for S1P in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay.

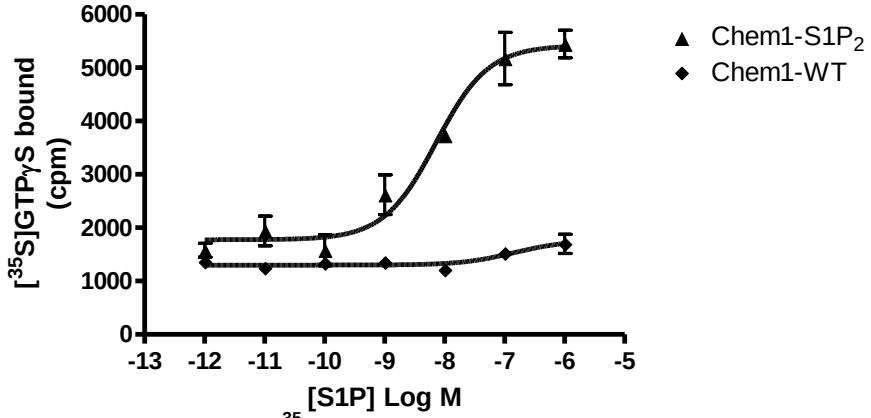

Figure 1. Binding of [^{35}S]-GTPγS to $S1P_2$ membrane preparation. 5 µg/well $S1P_2$ Membrane Preparation (catalog # HTS078M) was incubated with 0.1 nM [^{35}S]-GTPγS and increasing amounts of unlabeled S1P. Bound radioactivity was determined by filtration and scintillation counting.

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500
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SPECIFICATIONS: EC50 in GTPγS binding assay by S1P: ~ 7.2nM

Species: human EDG-5 cDNA encoding $S1P_2$ (Accession Number: NM_004230)

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 0.1 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 S1P is 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, 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 S1P -stimulated [^{35}S]-GTPγS binding.

The $S1P_2$ 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 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:

Anliker B and Chun J (2004) Lysophospholipid G Protein-coupled Receptors. *J. Biol. Chem.* 279: 20555-20558.

Arikawa K *et al.* (2003) Ligand-dependent Inhibition of B16 Melanoma Cell Migration and Invasion via Endogenous $S1P_2$ G Protein-coupled Receptor. *J. Biol. Chem.* 278: 32841-32851.

Goparaju SK *et al.* (2005) The $S1P_2$ Receptor Negatively Regulates Platelet-Derived Growth Factor-Induced Motility and Proliferation. *Mol. Cell. Biol.* 25: 4237-4249.

Kono M *et al.* (2004) The Sphingosine-1-phosphate Receptors $S1P_1$, $S1P_2$, and $S1P_3$ Function Coordinately during Embryonic Angiogenesis. J. Biol. Chem. 279: 29367-29373

Sugimoto N *et al.* (2003) Inhibitory and Stimulatory Regulation of Rac and Cell Motility by the $G_{12/13}$-Rho and G_i Pathways Integrated Downstream of a Single G Protein-Coupled Sphingosine-1-Phosphate Receptor Isoform. *Mol. Cell. Biol.* 23: 1534-1545.

Windh RT *et al.* (1999) Differential Coupling of the Sphingosine 1-Phosphate Receptors Edg-1, Edg-3, and H218/Edg-5 to the G_i, G_q, and G_{12} Families of Heterotrimeric G Proteins. *J. Biol. Chem.* 274: 27351-27358.

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 µL 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.*

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