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CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN $S1P_5$ LYSOPHOSPHOLIPID RECEPTOR

CATALOG NUMBER:	HTS193M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

Sphingosine 1-phosphate (S1P) is a biologically active lysophospholipid that transmits signals through a family of five G-protein-coupled receptors to regulate cell proliferation, migration, cytoskeletal organization, and differentiation (Spiegel and Milstien , 2003). $S1P_5$ can couple with Gi/o and G12/13, and it mediates S1P induced adenylate cyclase inhibition and Ca^{2+} mobilization like the other S1P receptors. However, unlike the other S1P receptors, it mediates inhibition of MAPK activation and cell proliferation (Im *et al*., 2000). $S1P_5$ is predominantly expressed in the white matter tracts and oligodendrocytes and is particularly abundant in the anterior commissure, corpus collosum, and optic tract (Terai *et al*., 2003). S1P induces process retraction in pre-oligodendrocytes and supports cell survival in mature oligodendrocytes by activating $S1P_5$, which indicates a role for $S1P_5$ in maturation and myelination of oligodendrocytes (Jaillard *et al*., 2005). Millipore's $S1P_5$ 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 $S1P_5$ interactions with its ligands. The membrane preparations exhibit EC50s of 2.4 nM for S1P in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay.

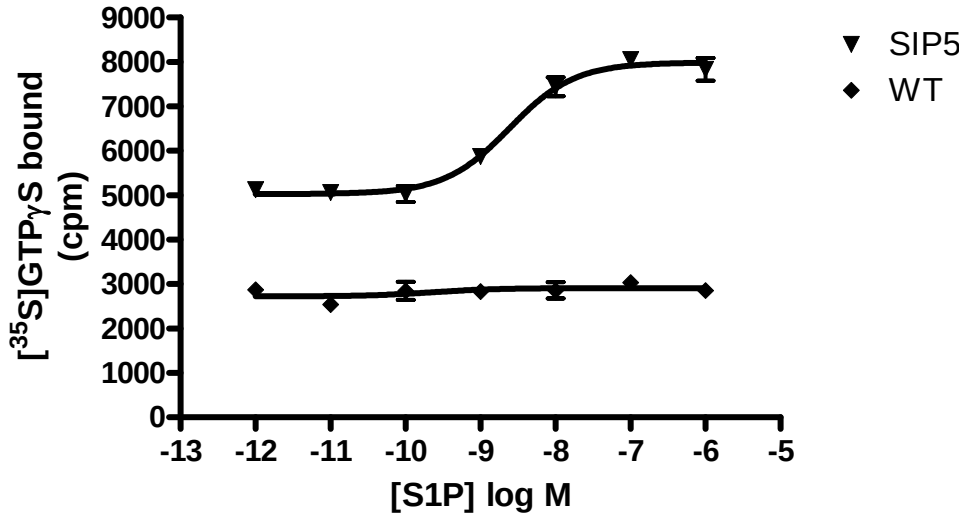

Figure 1. Binding of [^{35}S]-GTPγS to $S1P_5$ membrane preparation. 5 μg/well $S1P_5$ Membrane Preparation (catalog # HTS193M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled S1P. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: 1 unit = 5 μg
EC50 in GTPγS binding assay by S1P: ~ 2.4 nM

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Species: Full-length human EDG8 cDNA encoding $S1P_5$ (Accession Number: NM_030760)

HOST CELLS: Chem-5, an adherent cell line expressing a promiscuous G-protein.

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

The $S1P_5$ 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:

Im DS *et al*. (2000) Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. *J. Biol. Chem.* 275: 14281-6

Jaillard C *et al*. (2005) Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *J. Neurosci.* 25: 1459-1469.

Spiegel S and Milstien S. (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat. Rev. Mol. Cell Biol.* 4: 397-407.

Terai K *et al.* (2003) Edg-8 receptors are preferentially expressed in oligodendrocyte lineage cells of the rat CNS. *Neuroscience* 116: 1053-1062

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