

PRODUCT DATASHEET
ChemiScreen™ S1P₃ Lysophospholipid Membrane Preparation

CATALOG NUMBER:	HTS097M	QUANTITY:	200 units
LOT NUMBER:	SC20180725	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, S1P₁₋₅ (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). S1P₁ (Edg-1) signals exclusively through G_i, whereas S1P₂ (Edg-5) and S1P₃ (Edg-3) activate G_i, G_q and G_{12/13} (Windh *et al.*, 1999). Although S1P₁ and S1P₃ promote cell migration, S1P₂ 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 (Sugimoto *et al.*, 2003). Studies with knockout mice indicate that S1P₂ and S1P₃ have redundant functions in maintaining vascular integrity during embryonic development (Kono *et al.*, 2004). In addition, S1P₃ regulates immune responses by contributing to endothelial barrier function in splenic marginal zones (Girkontaite *et al.*, 2004). S1P₃ 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 S1P₃ interactions with its ligands. The membrane preparation exhibits EC₅₀ of 7.4nM for S1P in a GTP_γS binding assay.

APPLICATIONS: GTP_γS Assay.

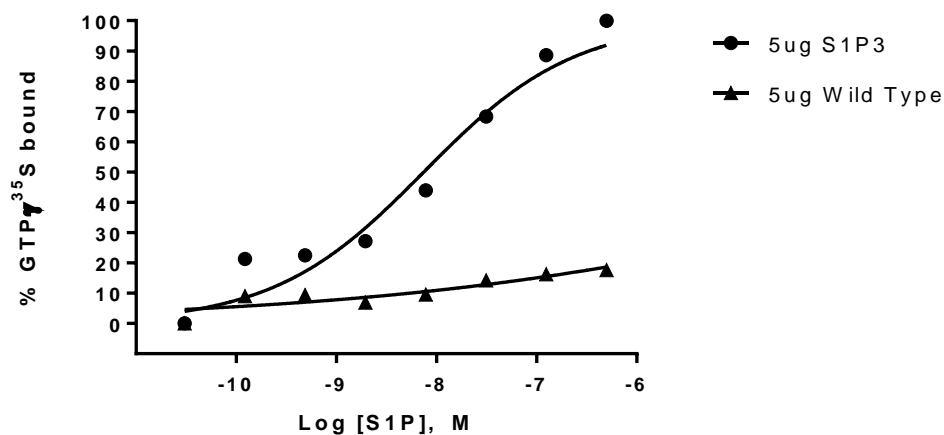


Figure 1. Binding of [³⁵S]-GTP_γS to S1P₃ membrane preparation. 5 μg/well S1P₃ Membrane Preparation (catalog # HTS097M) was incubated with 0.3 nM [³⁵S]-GTP_γS and increasing amounts of unlabeled S1P. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: EC50 in GTP γ S binding assay by S1P: ~ 7.4nM.

Species: Full-length human EDG3 cDNA encoding S1P₃ (Accession Number: NM_005226).

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

RECOMMENDED 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 0.1 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl₂/0.5 μ M GDP in a nonbinding 96-well plate. Unlabeled S1P is added to the final concentration indicated in Figure 1 (final volume 100 μ L), and incubated for 30 min at 30°C. The binding reaction is transferred to a GF/B filter plate (EMD 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 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 S1P -stimulated [³⁵S]-GTP γ S binding.

The S1P₃ 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:

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. Anliker B and Chun J (2004) Lysophospholipid G Protein-coupled Receptors. *J. Biol. Chem.* 279: 20555-20558.
2. Girkontaite I *et al.* (2004) The sphingosine-1-phosphate (S1P) lysophospholipid receptor S1P₃ regulates MAdCAM-1⁺ endothelial cells in splenic marginal sinus organization. *J. Exp. Med.* 200: 1491-501.
3. Kono M *et al.* (2004) The Sphingosine-1-phosphate Receptors S1P₁, S1P₂, and S1P₃ Function Coordinately during Embryonic Angiogenesis. *J. Biol. Chem.* 279: 29367-29373.
4. 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.
5. 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 G₁₂ Families of Heterotrimeric G Proteins. *J. Biol. Chem.* 274: 27351-27358.

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