



CHEMISCREEN™ MEMBRANE PREPARATION HUMAN RECOMBINANT S1P₁ LYSOPHOSPHOLIPID RECEPTOR

CATALOG NUMBER: HTS176M **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. S1P₁ was the first identified S1P receptor. It primarily couples to PTX-sensitive Gi/o proteins and mediates S1P-induced adenylate cyclase inhibition. Expression of S1P₁ is pervasive, including spleen, brain, heart, lung, adipose tissues, liver, thymus, kidney, and skeletal muscle (Zhang *et al.* 1999). The deletion of S1P₁ in mice results in embryonic lethality (Liu *et al.*, 2000) with death attributable to incomplete vascular maturation. Recent reports demonstrate specific roles for S1P₁ in lymphocyte recirculation/egress (Matloubian *et al.*, 2004). Millipore's S1P₁ 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 S1P₁ interactions with its ligands. The membrane preparation exhibits an EC₅₀ of 9.5 nM for Sphingosine 1-phosphate in a GTPγS binding assay.

APPLICATIONS: GTPγS Binding

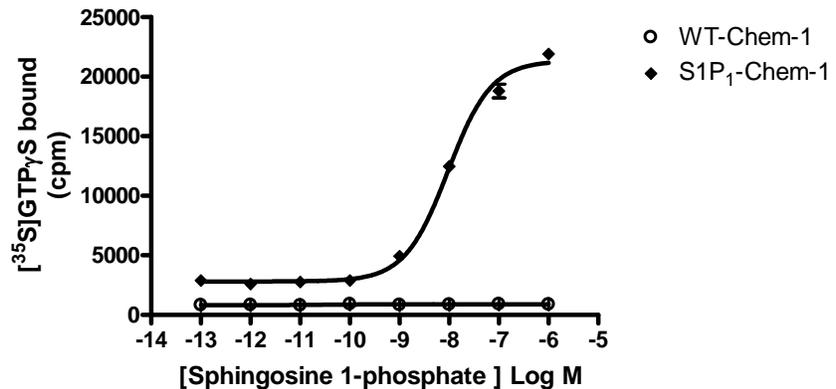


Figure 1. Binding of [³⁵S]-GTPγS to S1P₁ membrane preparation with high GDP concentration. 5 μg/well S1P₁ Membrane Preparation (catalog # HTS176M) or Wild-Type Chem-1 Membrane Preparation (Millipore catalog # HTS000MC1) was incubated with 0.3 nM [³⁵S]-GTPγS, 10 μM GDP and increasing amounts of unlabeled sphingosine 1-phosphate. Bound radioactivity was determined by filtration and scintillation counting.

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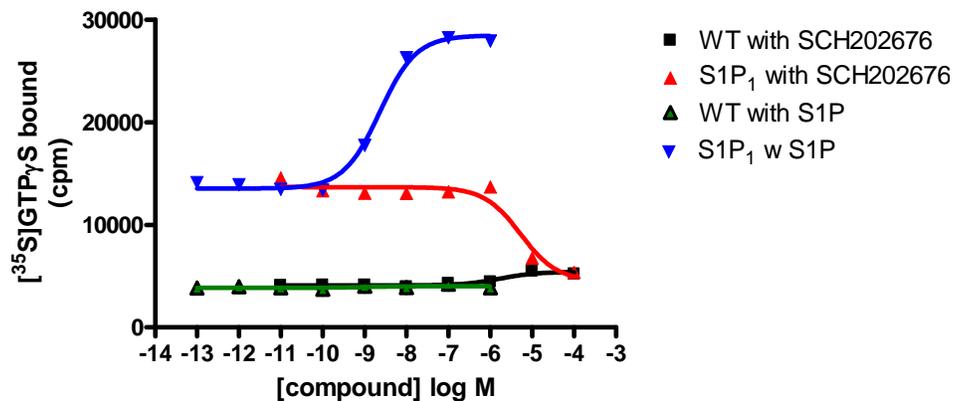


Figure 2. Binding of [³⁵S]-GTP_γS to S1P₁ membrane preparation with low GDP concentration. 5 μg/well S1P₁ Membrane Preparation (catalog # HTS176M) or Wild-Type Chem-1 Membrane Preparation (Millipore catalog # HTS000MC1) was incubated with 0.3 nM [³⁵S]-GTP_γS, 0.5 μM GDP and increasing amounts of unlabeled sphingosine 1-phosphate or SCH202676. Bound radioactivity was determined by filtration and scintillation counting. Increased constitutive activity of S1P₁ was observed with low GDP concentration, and this constitutive activity was inhibited by the broad spectrum GPCR inhibitor SCH202676.

SPECIFICATIONS: 1 unit = 5 μg

EC50 in GTP_γS binding assay by Sphingosine 1-phosphate: ~ 9.5 nM

Species: Full-length human EDG1 cDNA encoding S1P₁ (Accession Number: NM_001400)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G_{α15}

ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [³⁵S]-GTP_γS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl₂/10 μM GDP in a nonbinding 96-well plate. Unlabeled Sphingosine 1-phosphate 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.

Note: Performing the reaction with lower GDP concentrations (0.5 μM) results in elevated constitutive activity (Figure 2).

PRESENTATION:

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 [³⁵S]-GTP_γS binding.

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 the indicated concentration 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.



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REFERENCES:

Zhang GF *et al.* (1999) Comparative analysis of three murine G-protein coupled receptors activated by sphingosine-1-phosphate. *Gene* 227: 89–99.

Liu Y *et al.* (2000) Edg-1, the G protein–coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J. Clin. Invest.* 106, 951–961

Matloubian M *et al.* (2004) Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 427: 355–360.

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