

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT S1P₂ LYSOPHOSPHOLIPID RECEPTOR**

CATALOG NUMBER:	HTS078C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/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 (Arikawa *et al.*, 2003; Sugimoto *et al.*, 2003; Goparaju *et al.*, 2005). Studies with knockout mice indicate that S1P₂ and S1P₃ have redundant functions in maintaining vascular integrity during embryonic development (Kono *et al.*, 2004). Chemicon's cloned human S1P₂-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant S1P₂ expression on the cell surface and contains high levels of the promiscuous G protein G α 15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between S1P₂ and its ligands

APPLICATIONS: Calcium flux assay, ligand binding assays

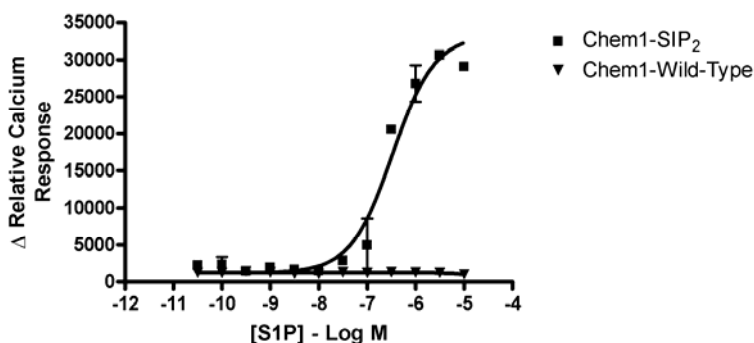


Figure 1. Calcium flux in S1P₂-expressing Chem-1 cell line induced by S1P. S1P₂-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to S1P (10⁻⁵ to 10^{-10.5} M) was determined in triplicate on a Molecular Devices Flex Station. An increase of greater than 20,000 fluorescence units was obtained.

SPECIFICATIONS: EC50 for calcium mobilization by S1P: ~ 300 nM

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

TRANSFECTION: Full-length human EDG-5 cDNA encoding S1P₂ (Accession Number: NM_004230)

GROWTH MEDIA: DMEM containing 4.5 g/L glucose/10% heat inactivated fetal bovine serum/1x nonessential amino acids/10 mM HEPES/0.25 mg/mL Geneticin (G418)/100 U/mL each penicillin and streptomycin

PRESENTATION: Cells are frozen at 2×10^6 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING: Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing 20 mL growth media, and place in a humidified 37°C incubator with 5% CO₂. After 8-24 h, cells will adhere to the plate, at which time the media should be replaced to remove residual DMSO. Cells are passaged by washing with Ca⁺⁺ and Mg⁺⁺-free HBSS (10 mL/T75), incubating with 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) for 5-10 minutes at 37°C, and rapping the side of the flask to dislodge the cells. Neutralize the trypsin by addition of 4 volumes growth media. Cells are typically passaged 1:10 with every 3-4 days, and should be passaged at least once after thawing prior to use in calcium flux assays.

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 S1P₂ G Protein-coupled Receptor. *J. Biol. Chem.* 278: 32841-32851.

Goparaju SK *et al.* (2005) The S1P₂ 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 S1P₁, S1P₂, and S1P₃ 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 G₁₂ Families of Heterotrimeric G Proteins. *J. Biol. Chem.* 274: 27351-27358.

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HUMAN RECOMBINANT SIP₂ LYSOPHOSPHOLIPID RECEPTOR**

Product No. HTS078C

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