

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT S1P₃ LYSOPHOSPHOLIPID RECEPTOR**

CATALOG NUMBER:	HTS097C	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 (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). Millipore's cloned human S1P₃-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant S1P₃ expression on the cell surface and contains high levels of the promiscuous G protein G_α15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between S1P₃ and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

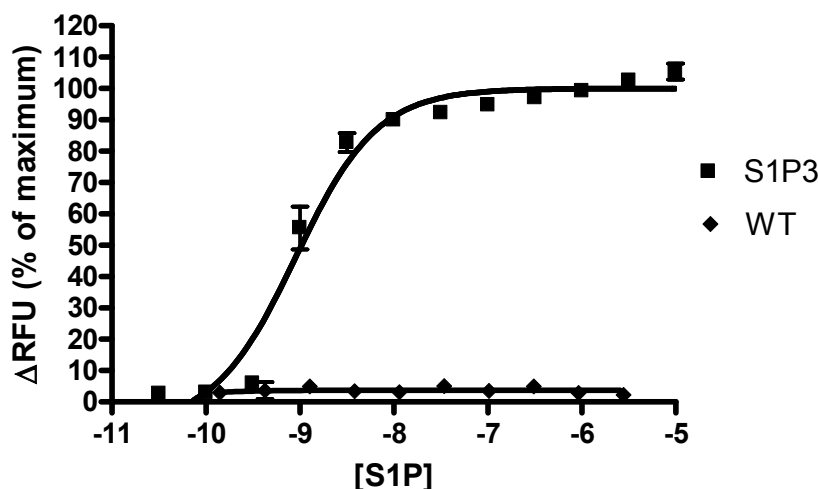


Figure 1. Calcium flux in S1P₃-expressing Chem-1 cell line induced by S1P. S1P₃-expressing Chem-1 cells and Wild-Type Chem-1 cells (Millipore catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to recombinant human S1P (10^{-5.5} to 10⁻¹⁰ M) was determined in triplicate on a Molecular Devices FLIPR^{TETRA}.

SPECIFICATIONS: EC50 for calcium mobilization by S1P: ~ 0.9 nM

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

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

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:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. 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 growth media. Place the flask in a humidified incubator at 37°C with 5% CO₂.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca⁺⁺ and Mg⁺⁺ (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO₂ until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5×10^6 cells/mL in Chem-1 Freezing Media (cell densities of $2-10 \times 10^6$ are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Chem-1 Plating Media for plating for calcium assay.

MEDIA:

Chem-1 Growth Media:

- DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020)
- 10% heat-inactivated FBS
- 1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
- 10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
- 100 U/mL penicillin and streptomycin (from 100x stock, Millipore TMS-AB2-C)
- 250µg/mL Genetecin/G-418

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Chem-1 Plating Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

Chem-1 Freezing Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
20% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep
10% DMSO (cell culture grade)

REFERENCES:

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

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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.

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