

**READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS  
HUMAN RECOMBINANT S1P<sub>1</sub> LYSOPHOSPHOLIPID RECEPTOR**

<b>CATALOG NUMBER:</b>	HTS176F	<b>QUANTITY:</b>	1 vial, 1 mL
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	1 x 10 <sup>7</sup> viable cells/mL

**BACKGROUND:** Millipore's Ready-To-Assay™ Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay™ cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay™ cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay™ cells is identical to that of the originating GPCR cell line.

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. S1P<sub>1</sub> was the first identified S1P receptor. It primarily couples to PTX-sensitive Gi/o proteins and mediates S1P-induced adenylate cyclase inhibition. Expression of S1P<sub>1</sub> is pervasive, including spleen, brain, heart, lung, adipose tissues, liver, thymus, kidney, and skeletal muscle (Zhang *et al.* 1999). The deletion of S1P<sub>1</sub> in mice results in embryonic lethality (Liu *et al.*, 2000) with death attributable to incomplete vascular maturation. Recent reports demonstrate specific roles for S1P<sub>1</sub> in lymphocyte recirculation/egress (Matloubian *et al.*, 2004). Millipore's cloned human S1P<sub>1</sub>-expressing cell line is made in the Chem-4 host, an adherent cell line that supports high levels of recombinant S1P<sub>1</sub> expression on the cell surface and contains high levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. The untreated S1P<sub>1</sub>-Chem-4 cell line and the Ready-To-Assay™ S1P<sub>1</sub> cells have equivalent EC50s for S1P.

**APPLICATIONS:** Calcium flux assay

**SPECIFICATIONS:**

	EC50 for S1P (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	2.04	1695	0.58
Continuous Passage Cells	0.72	2050	0.50

**HOST CELLS:** Chem-4, an adherent cell line expressing a recombinant promiscuous G-protein.

TRANSFECTION: Full-length human EDG1 cDNA encoding S1P<sub>1</sub> (Accession Number: NM\_001400)

PLATING MEDIA:

DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)  
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 Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

PRESENTATION:

Cells are frozen at  $1 \times 10^7$  cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

STORAGE:

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

ASSAY PROTOCOL:

- 1) 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.
- 2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
- 3) Resuspend cells to  $0.5 \times 10^6$  cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200  $\mu$ L per well to obtain a density of approximately  $1 \times 10^5$  cells/well.
- 4) Place the assay plate in a humidified 37°C incubator with 5% CO<sub>2</sub>.
- 5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

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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