

**READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT S1P₄ LYSOPHOSPHOLIPID RECEPTOR**

CATALOG NUMBER:	HTS192F	QUANTITY:	1 vial, 1 mL
LOT NUMBER:		CONCENTRATION:	1 x 10 ⁷ 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 (Spiegel and Milstien, 2003). S1P₄ receptor was reported to couple to multiple subsets of heterotrimeric G proteins (including Gi/o, and G12/13, and possibly Gs). Unlike other S1P receptors, S1P₄ expression is restricted in human and mouse to lymph node, spleen, lung, and thymus (Ishii *et al.*, 2001). This expression pattern suggests potential roles of S1P₄ in the immune system. Millipore's cloned S1P₄-expressing cell line is made in the Chem-5 host, an adherent cell line that supports high levels of recombinant S1P₄ expression on the cell surface and contains high levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. The untreated S1P₄-Chem-5 cell line and the Ready-To-Assay™ S1P₄ cells have equivalent EC50s for S1P.

APPLICATIONS: Calcium flux assay

SPECIFICATIONS:

	EC50 for S1P (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	29.19	3251	0.79
Continuous Passage Cells	16.0	2584	0.76

HOST CELLS: Chem-5, an adherent cell line expressing a recombinant promiscuous G-protein.

TRANSFECTION: Full-length human EDG6 cDNA encoding S1P₄ (Accession Number: NM_003775)

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 1×10^7 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 0.5×10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 μ L per well to obtain a density of approximately 1×10^5 cells/well.
- 4) Place the assay plate in a humidified 37°C incubator with 5% CO₂.
- 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:

Spiegel S and Milstien S. (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat. Rev. Mol. Cell Biol.* 4(5):397-407.

Ishii I, Friedman B, Ye X, Kawamura S, McGiffert C, Contos JJ, Kingsbury MA, Zhang G, Brown JH, Chun J. (2001) Selective loss of sphingosine 1-phosphate signaling with no obvious phenotypic abnormality in mice lacking its G protein-coupled receptor, LP(B3)/EDG-3. *J. Biol. Chem.* 276(36):33697-704

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HUMAN RECOMBINANT S1P₄ LYOPHOSPHOLIPID RECEPTOR
Product No. HTS192F**

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