eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ S_1P_2 Lysophospholipid Receptor Frozen Cells

CATALOG NUMBER: HTS078RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

Sphingosine 1-phosphate (S1P) is a bioactive lipid that binds to and activates a family of GPCRs, $S_1P_{1\text{-}5}$ (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). $S1P_1$ (Edg-1) signals exclusively through G_i, whereas S_1P_2 (Edg-5) and S_1P_3 (Edg-3) activate G_i, G_q and $G_{12/13}$ (Windh *et al.*, 1999). Although S_1P_1 and S_1P_3 promote cell migration, S_1P_2 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 S_1P_2 and S_1P_3 have redundant functions in maintaining vascular integrity during embryonic development (Kono *et al.*, 2004). Cloned human S_1P_2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant S_1P_2 expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at S_1P_2.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

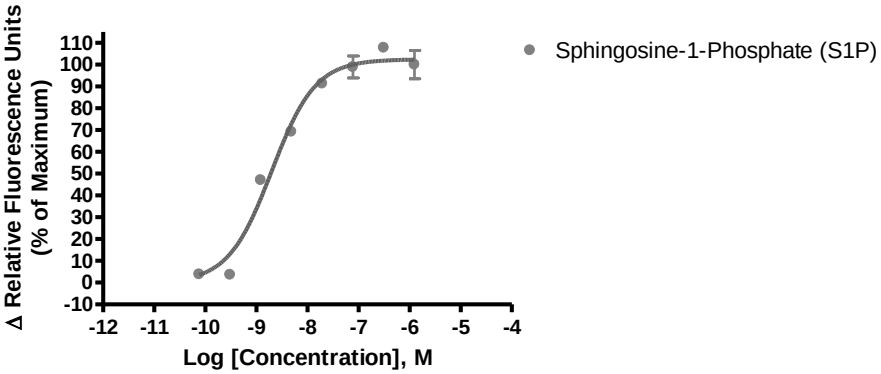

Figure 1. Representative data for activation of S_1P_2 receptor. Calcium flux in S_1P_2 –expressing Chem-1 cell line induced by S1P. S_1P_2 –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 5,000 RLU (Relative Light Units).

Table 1. EC_{50} value of S_1P_2 -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
S1P	Calcium Flux	2	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
S1P ligand	Sigma: S9666
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for $FLIPR^{TETRA}$® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein

EXONGENOUS GENE EXPRESSION

EDG5 cDNA (Accession Number: NM_004230; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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  1 - ATG GGC AGC TTG TAC TCG GAG TAC CTG AAC CCC AAC AAG GTC CAG GAA CAC TAT AAT TAT ACC AAG GAG ACG  - 72
  1 -  M   G   S   L   Y   S   E   Y   L   N   P   N   K   V   Q   E   H   Y   N   Y   T   K   E   T  - 24

 73 - CTG GAA ACG CAG GAG ACG ACC TCC CGC CAG GTG GCC TCG GCC TTC ATC GTC ATC CTC TGT TGC GCC ATT GTG  - 144
 25 -  L   E   T   Q   E   T   T   S   R   Q   V   A   S   A   F   I   V   I   L   C   C   A   I   V  - 48

145 - GTG GAA AAC CTT CTG GTG CTC ATT GCG GTG GCC CGA AAC AGC AAG TTC CAC TCG GCA ATG TAC CTG TTT CTG  - 216
 49 -  V   E   N   L   L   V   L   I   A   V   A   R   N   S   K   F   H   S   A   M   Y   L   F   L  - 72

217 - GGC AAC CTG GCC GCC TCC GAT CTA CTG GCA GGC GTG GCC TTC GTA GCC AAT ACC TTG CTC TCT GGC TCT GTC  - 288
 73 -  G   N   L   A   A   S   D   L   L   A   G   V   A   F   V   A   N   T   L   L   S   G   S   V  - 96

289 - ACG CTG AGG CTG ACG CCT GTG CAG TGG TTT GCC CGG GAG GGC TCT GCC TTC ATC ACG CTC TCG GCC TCT GTC  - 360
 97 -  T   L   R   L   T   P   V   Q   W   F   A   R   E   G   S   A   F   I   T   L   S   A   S   V  - 120

361 - TTC AGC CTC CTG GCC ATC GCC ATT GAG CGC CAC GTG GCC ATT GCC AAG GTC AAG CTG TAT GGC AGC GAC AAG  - 432
121 -  F   S   L   L   A   I   A   I   E   R   H   V   A   I   A   K   V   K   L   Y   G   S   D   K  - 144

433 - AGC TGC CGC ATG CTT CTG CTC ATC GGG GCC TCG TGG CTC ATC TCG CTG GTC CTC GGT GGC CTG CCC ATC CTT  - 504
145 -  S   C   R   M   L   L   L   I   G   A   S   W   L   I   S   L   V   L   G   G   L   P   I   L  - 168

505 - GGC TGG AAC TGC CTG GGC CAC CTC GAG GCC TGC TCC ACT GTC CTG CCT CTC TAC GCC AAG CAT TAT GTG CTG  - 576
169 -  G   W   N   C   L   G   H   L   E   A   C   S   T   V   L   P   L   Y   A   K   H   Y   V   L  - 192

577 - TGC GTG GTG ACC ATC TTC TCC ATC ATC CTG TTG GCC ATC GTG GCC CTG TAC GTG CGC ATC TAC TGC GTG GTC  - 648
193 -  C   V   V   T   I   F   S   I   I   L   L   A   I   V   A   L   Y   V   R   I   Y   C   V   V  - 216

649 - CGC TCA AGC CAC GCT GAC ATG GCC GCC CCG CAG ACG CTA GCC CTG CTC AAG ACG GTC ACC ATC GTG CTA GGC  - 720
217 -  R   S   S   H   A   D   M   A   A   P   Q   T   L   A   L   L   K   T   V   T   I   V   L   G  - 240

721 - GTC TTT ATC GTC TGC TGG CTG CCC GCC TTC AGC ATC CTC CTT CTG GAC TAT GCC TGT CCC GTC CAC TCC TGC  - 792
241 -  V   F   I   V   C   W   L   P   A   F   S   I   L   L   L   D   Y   A   C   P   V   H   S   C  - 264

793 - CCG ATC CTC TAC AAA GCC CAC TAC TTT TTC GCC GTC TCC ACC CTG AAT TCC CTG CTC AAC CCC GTC ATC TAC  - 864
265 -  P   I   L   Y   K   A   H   Y   F   F   A   V   S   T   L   N   S   L   L   N   P   V   I   Y  - 288

865 - ACG TGG CGC AGC CGG GAC CTG CGG CGG GAG GTG CTT CGG CCG CTG CAG TGC TGG AGG CCG GGG GTG GGG GTG  - 936
289 -  T   W   R   S   R   D   L   R   R   E   V   L   R   P   L   Q   C   W   R   P   G   V   G   V  - 312

937 - CAA GGA CGG AGG CGG GGC GGG ACC CCG GGC CAC CAC CTC CTG CCA CTC CGC AGC TCC AGC TCC CTG GAG AGG  - 1008
313 -  Q   G   R   R   R   G   G   T   P   G   H   H   L   L   P   L   R   S   S   S   S   L   E   R  - 336

1009 - GGC ATG CAC ATG CCC ACG TCA CCC ACG TTT CTG GAG GGC AAC ACG GTG GTC TGA
337 -  G   M   H   M   P   T   S   P   T   F   L   E   G   N   T   V   V  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS078M	ChemiScreen™ S_1P_2 Lysophospholipid membrane preps
HTS078LT	ChemiBrite™ S_1P_2 Lysophospholipid frozen cells

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3. Goparaju SK et al. (2005) The S1P2 Receptor Negatively Regulates Platelet-Derived Growth Factor-Induced Motility and Proliferation. Mol. Cell. Biol. 25: 4237-4249.
4. 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

5. Sugimoto N et al. (2003) Inhibitory and Stimulatory Regulation of Rac and Cell Motility by the G12/13-Rho and Gi Pathways Integrated Downstream of a Single G Protein-Coupled Sphingosine-1-Phosphate Receptor Isoform. Mol. Cell. Biol. 23: 1534-1545.
6. Windh RT et al. (1999) Differential Coupling of the Sphingosine 1-Phosphate Receptors Edg-1, Edg-3, and H218/Edg-5 to the Gi, Gq, and G12 Families of Heterotrimeric G Proteins. J. Biol. Chem. 274: 27351-27358.

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