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PRODUCT DATASHEET

Ready-to-Assay™ S_1P_3 Lysophospholipid receptors Receptor Frozen Cells

CATALOG NUMBER: HTS097RTA

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, $S1P_{1\text{-}5}$ (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 $S1P_2$ (Edg-5) and $S1P_3$ (Edg-3) activate G_i, G_q and $G_{12/13}$ (Windh *et al.*, 1999). Although $S1P_1$ and $S1P_3$ promote cell migration, $S1P_2$ 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 (Sugimoto *et al.*, 2003). Studies with knockout mice indicate that $S1P_2$ and $S1P_3$ have redundant functions in maintaining vascular integrity during embryonic development (Kono *et al.*, 2004). In addition, $S1P_3$ regulates immune responses by contributing to endothelial barrier function in splenic marginal zones (Girkontaite *et al.*, 2004). Cloned human S_1P_3 expressing cell line is made in the Chem-1 host, which supports high levels of recombinant S_1P_3 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at S_1P_3.

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.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

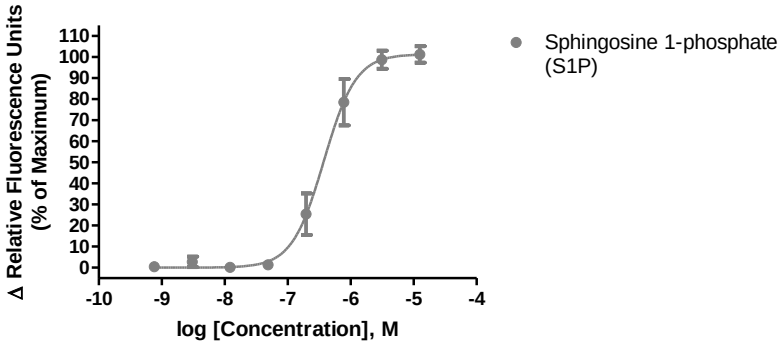

Figure 1. Representative data for activation of S_1P_3 receptor. Calcium flux in S_1P_3–expressing Chem-1 cell line induced by Sphingosine 1-phosphate (S1P). S_1P_3–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 27,000 RLU (Relative Light Units).

Table 1. EC_{50} value of S_1P_3-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Sphingosine 1-phosphate (S1P)	Calcium Flux	0.85	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
Sphingosine 1-phosphate (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

EXONGENOUS GENE EXPRESSION

EDG3 / S1PR3 cDNA (Accession Number: NM_005226; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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   1 - ATG GCA ACT GCC CTC CCG CCG CGT CTC CAG CCG GTG CGG GGG AAC GAG ACC CTG CGG GAG  - 60
   1 -  M   A   T   A   L   P   P   R   L   Q   P   V   R   G   N   E   T   L   R   E  - 20

  61 - CAT TAC CAG TAC GTG GGG AAG TTG GCG GGC AGG CTG AAG GAG GCC TCC GAG GGC AGC ACG  - 120
  21 -  H   Y   Q   Y   V   G   K   L   A   G   R   L   K   E   A   S   E   G   S   T  - 40

 121 - CTC ACC ACC GTG CTC TTC TTG GTC ATC TGC AGC TTC ATC GTC TTG GAG AAC CTG ATG GTT  - 180
  41 -  L   T   T   V   L   F   L   V   I   C   S   F   I   V   L   E   N   L   M   V  - 60

 181 - TTG ATT GCC ATC TGG AAA AAC AAT AAA TTT CAC AAC CGC ATG TAC TTT TTC ATT GGC AAC  - 240
  61 -  L   I   A   I   W   K   N   N   K   F   H   N   R   M   Y   F   F   I   G   N  - 80

 241 - CTG GCT CTC TGC GAC CTG CTG GCC GGC ATC GCT TAC AAG GTC AAC ATT CTG ATG TCT GGC  - 300
  81 -  L   A   L   C   D   L   L   A   G   I   A   Y   K   V   N   I   L   M   S   G  - 100

 301 - AAG AAG ACG TTC AGC CTG TCT CCC ACG GTC TGG TTC CTC AGG GAG GGC AGT ATG TTC GTG  - 360
 101 -  K   K   T   F   S   L   S   P   T   V   W   F   L   R   E   G   S   M   F   V  - 120

 361 - GCC CTT GGG GCG TCC ACC TGC AGC TTA CTG GCC ATC GCC ATC GAG CGG CAC TTG ACA ATG  - 420
 121 -  A   L   G   A   S   T   C   S   L   L   A   I   A   I   E   R   H   L   T   M  - 140

 421 - ATC AAA ATG AGG CCT TAC GAC GCC AAC AAG AGG CAC CGC GTC TTC CTC CTG ATC GGG ATG  - 480
 141 -  I   K   M   R   P   Y   D   A   N   K   R   H   R   V   F   L   L   I   G   M  - 160

 481 - TGC TGG CTC ATT GCC TTC ACG CTG GGC GCC CTG CCC ATT CTG GGC TGG AAC TGC CTG CAC  - 540
 161 -  C   W   L   I   A   F   T   L   G   A   L   P   I   L   G   W   N   C   L   H  - 180

 541 - AAT CTC CCT GAC TGC TCT ACC ATC CTG CCC CTC TAC TCC AAG AAG TAC ATT GCC TTC TGC  - 600
 181 -  N   L   P   D   C   S   T   I   L   P   L   Y   S   K   K   Y   I   A   F   C  - 200

 601 - ATC AGC ATC TTC ACG GCC ATC CTG GTG ACC ATC GTG ATC CTC TAC GCA CGC ATC TAC TTC  - 660
 201 -  I   S   I   F   T   A   I   L   V   T   I   V   I   L   Y   A   R   I   Y   F  - 220

 661 - CTG GTG AAG TCC AGC AGC CGT AAG GTG GCC AAC CAC AAC AAC TCG GAG CGG TCC ATG GCA  - 720
 221 -  L   V   K   S   S   S   R   K   V   A   N   H   N   N   S   E   R   S   M   A  - 240

 721 - CTG CTG CGG ACC GTG GTG ATT GTG GTG AGC GTG TTC ATC GCC TGC TGG TCC CCA CTC TTC  - 780
 241 -  L   L   R   T   V   V   I   V   V   S   V   F   I   A   C   W   S   P   L   F  - 260

 781 - ATC CTC TTC CTC ATT GAT GTG GCC TGC AGG GTG CAG GCG TGC CCC ATC CTC TTC AAG GCT  - 840
 261 -  I   L   F   L   I   D   V   A   C   R   V   Q   A   C   P   I   L   F   K   A  - 280

 841 - CAG TGG TTC ATC GTG TTG GCT GTG CTC AAC TCC GCC ATG AAC CCG GTC ATC TAC ACG CTG  - 900
 281 -  Q   W   F   I   V   L   A   V   L   N   S   A   M   N   P   V   I   Y   T   L  - 300

 901 - GCC AGC AAG GAG ATG CGG CGG GCC TTC TTC CGT CTG GTC TGC AAC TGC CTG GTC AGG GGA  - 960
 301 -  A   S   K   E   M   R   R   A   F   F   R   L   V   C   N   C   L   V   R   G  - 320

 961 - CGG GGG GCC CGC GCC TCA CCC ATC CAG CCT GCG CTC GAC CCA AGC AGA AGT AAA TCA AGC  - 1020
 321 -  R   G   A   R   A   S   P   I   Q   P   A   L   D   P   S   R   S   K   S   S  - 340

1021 - AGC AGC AAC AAT AGC AGC CAC TCT CCG AAG GTC AAG GAA GAC CTG CCC CAC ACA GCC CCC  - 1080
 341 -  S   S   N   N   S   S   H   S   P   K   V   K   E   D   L   P   H   T   A   P  - 360

1081 - TCA TCC TGC ATC ATG GAC AAG AAC GCA GCA CTT CAG AAT GGG ATC TTC TGC AAC TGA    - 1137
 361 -  S   S   C   I   M   D   K   N   A   A   L   Q   N   G   I   F   C   N   *    - 380
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS097M	ChemiScreen™ S_1P_3 Lysophospholipid receptor membrane prep

REFERENCES

1. Anliker B and Chun J (2004) Lysophospholipid G Protein-coupled Receptors. J. Biol. Chem. 279: 20555-20558.
2. Girkontaite I et al. (2004) The sphingosine-1-phosphate (S1P) lysophospholipid receptor S1P3 regulates MAdCAM-1+ endothelial cells in splenic marginal sinus organization. J. Exp. Med. 200: 1491-501.
3. 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.
4. 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.
5. 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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