eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ $S1P_4$ Lysophospholipid Receptor Frozen Cells

CATALOG NUMBER: HTS192RTA

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 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). $S1P_4$ receptor was reported to couple to multiple subsets of heterotrimeric G proteins (including $G_{i/o}$, and $G_{12/13}$, and possibly G_s). Unlike other S1P receptors, $S1P_4$ 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 $S1P_4$ in the immune system. Cloned human $S1P_4$-expressing cell line is made in the Chem-5 host, which supports high levels of recombinant $S1P_4$ expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at $S1P_4$.

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

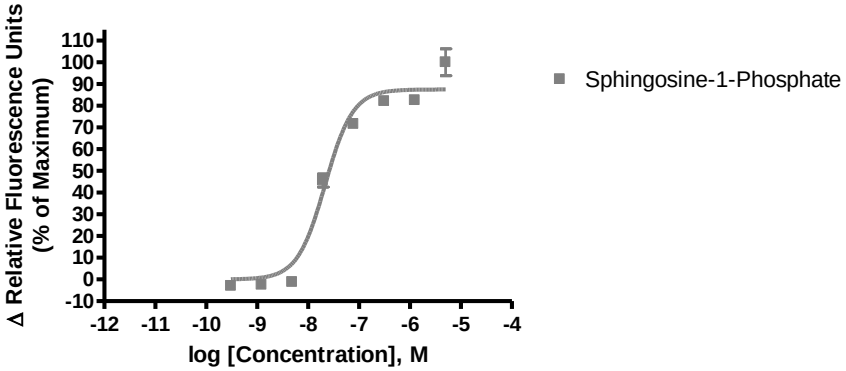

Figure 1. Representative data for activation of $S1P_4$ receptor. Calcium flux in $S1P_4$ –expressing Chem-5 cell line induced by SIP. $S1P_4$ –expressing Chem-5 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s) was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 800 RLU (Relative Light Units).

Table 1. EC_{50} values of $S1P_4$ -expressing Chem-5 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Sphingosine-1-Phosphate	Calcium Flux	22	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 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-5, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein and a proprietary promiscuous Gα protein

EXONGENOUS GENE EXPRESSION

EDG6 / S1PR4 cDNA (Accession Number: NM_003775; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG AAC GCC ACG GGG ACC CCG GTG GCC CCC GAG TCC
                                                 M   N   A   T   G   T   P   V   A   P   E   S

TGC CAA CAG CTG GCG GCC GGC GGG CAC AGC CGG CTC ATT GTT CTG CAC TAC AAC CAC TCG GGC CGG CTG
 C   Q   Q   L   A   A   G   G   H   S   R   L   I   V   L   H   Y   N   H   S   G   R   L

GCC GGG CGC GGG GGG CCG GAG GAT GGC GGC CTG GGG GCC CTG CGG GGG CTG TCG GTG GCC GCC AGC TGC
 A   G   R   G   G   P   E   D   G   G   L   G   A   L   R   G   L   S   V   A   A   S   C

CTG GTG GTG CTG GAG AAC TTG CTG GTG CTG GCG GCC ATC ACC AGC CAC ATG CGG TCG CGA CGC TGG GTC
 L   V   V   L   E   N   L   L   V   L   A   A   I   T   S   H   M   R   S   R   R   W   V

TAC TAT TGC CTG GTG AAC ATC ACG CTG AGT GAC CTG CTC ACG GGC GCG GCC TAC CTG GCC AAC GTG CTG
 Y   Y   C   L   V   N   I   T   L   S   D   L   L   T   G   A   A   Y   L   A   N   V   L

CTG TCG GGG GCC CGC ACC TTC CGT CTG GCG CCC GCC CAG TGG TTC CTA CGG GAG GGC CTG CTC TTC ACC
 L   S   G   A   R   T   F   R   L   A   P   A   Q   W   F   L   R   E   G   L   L   F   T

GCC CTG GCC GCC TCC ACC TTC AGC CTG CTC TTC ACT GCA GGG GAG CGC TTT GCC ACC ATG GTG CGG CCG
 A   L   A   A   S   T   F   S   L   L   F   T   A   G   E   R   F   A   T   M   V   R   P

GTG GCC GAG AGC GGG GCC ACC AAG ACC AGC CGC GTC TAC GGC TTC ATC GGC CTC TGC TGG CTG CTG GCC
 V   A   E   S   G   A   T   K   T   S   R   V   Y   G   F   I   G   L   C   W   L   L   A

GCG CTG CTG GGG ATG CTG CCT TTG CTG GGC TGG AAC TGC CTG TGC GCC TTT GAC CGC TGC TCC AGC CTT
 A   L   L   G   M   L   P   L   L   G   W   N   C   L   C   A   F   D   R   C   S   S   L

CTG CCC CTC TAC TCC AAG CGC TAC ATC CTC TTC TGC CTG GTG ATC TTC GCC GGC GTC CTG GCC ACC ATC
 L   P   L   Y   S   K   R   Y   I   L   F   C   L   V   I   F   A   G   V   L   A   T   I

ATG GGC CTC TAT GGG GCC ATC TTC CGC CTG GTG CAG GCC AGC GGG CAG AAG GCC CCA CGC CCA GCG GCC
 M   G   L   Y   G   A   I   F   R   L   V   Q   A   S   G   Q   K   A   P   R   P   A   A

CGC CGC AAG GCC CGC CGC CTG CTG AAG ACG GTG CTG ATG ATC CTG CTG GCC TTC CTG GTG TGC TGG GGC
 R   R   K   A   R   R   L   L   K   T   V   L   M   I   L   L   A   F   L   V   C   W   G

CCA CTC TTC GGG CTG CTG CTG GCC GAC GTC TTT GGC TCC AAC CTC TGG GCC CAG GAG TAC CTG CGG GGC
 P   L   F   G   L   L   L   A   D   V   F   G   S   N   L   W   A   Q   E   Y   L   R   G

ATG GAC TGG ATC CTG GCC CTG GCC GTC CTC AAC TCG GCG GTC AAC CCC ATC ATC TAC TCC TTC CGC AGC
 M   D   W   I   L   A   L   A   V   L   N   S   A   V   N   P   I   I   Y   S   F   R   S

AGG GAG GTG TGC AGA GCC GTG CTC AGC TTC CTC TGC TGC GGG TGT CTC CGG CTG GGC ATG CGA GGG CCC
 R   E   V   C   R   A   V   L   S   F   L   C   C   G   C   L   R   L   G   M   R   G   P

GGG GAC TGC CTG GCC CGG GCC GTC GAG GCT CAC TCC GGA GCT TCC ACC ACC GAC AGC TCT CTG AGG CCA
 G   D   C   L   A   R   A   V   E   A   H   S   G   A   S   T   T   D   S   S   L   R   P

AGG GAC AGC TTT CGC GGC TCC CGC TCG CTC AGC TTT CGG ATG CGG GAG CCC CTG TCC AGC ATC TCC AGC
 R   D   S   F   R   G   S   R   S   L   S   F   R   M   R   E   P   L   S   S   I   S   S

GTG CGG AGC ATC TGA
 V   R   S   I  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

Note: Chem-5 cells are derived from Chem-1 cells.

REFERENCES

1. Spiegel S and Milstien S. (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat. Rev. Mol. Cell Biol.* 4: 397-407.
2. 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: 33697-704

User Agreement (Label License)

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