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PRODUCT DATASHEET

Ready-to-Assay™ S_1P_1 Lysophospholipid Receptor Frozen Cells

CATALOG NUMBER: HTS176RTA

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. $S1P_1$ primarily couples to PTX-sensitive Gi/o proteins and mediates S1P-induced adenylate cyclase inhibition. Expression of $S1P_1$ is pervasive, including spleen, brain, heart, lung, adipose tissues, liver, thymus, kidney, and skeletal muscle (Zhang *et al.* 1999). The deletion of $S1P_1$ in mice results in embryonic lethality (Liu *et al.*, 2000) with death attributable to incomplete vascular maturation. Recent reports demonstrate specific roles for $S1P_1$ in lymphocyte recirculation/egress (Matloubian *et al.,* 2004). Cloned human S_1P_1-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant S_1P_1expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein 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_1.

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

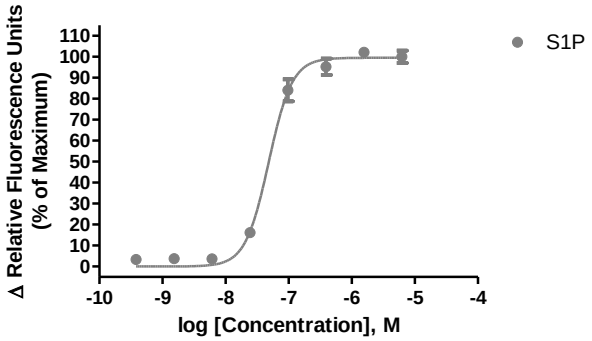

Figure 1. Representative data for activation of S_1P_1 receptor. Calcium flux in S_1P_1 –expressing Chem-4 cell line induced by Sphigosine-1-Phosphate (S1P) S_1P_1 –expressing Chem-4 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution 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 22,000 RLU (Relative Light Units).

Table 1. Summary of EC_{50} value of S_1P_1-expressing Chem-4 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Sphingosine-1-Phosphate	Calcium Flux	48	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-1, an adherent rat hematopoietic cell line expressing endogenous G‧15 protein as well as an exogenous proprietary promiscuous Gα protein.

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EXONGENOUS GENE EXPRESSION

EDG1 / S1PR1 cDNA (Accession Number: NM_001400; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                        ATG GGG CCC ACC AGC GTC CCG CTG GTC AAG GCC CAC CGC AGC
                                         M   G   P   T   S   V   P   L   V   K   A   H   R   S

TCG GTC TCT GAC TAC GTC AAC TAT GAT ATC ATC GTC CGG CAT TAC AAC TAC ACG GGA AAG CTG AAT ATC
 S   V   S   D   Y   V   N   Y   D   I   I   V   R   H   Y   N   Y   T   G   K   L   N   I

AGC GCG GAC AAG GAG AAC AGC ATT AAA CTG ACC TCG GTG GTG TTC ATT CTC ATC TGC TGC TTT ATC ATC
 S   A   D   K   E   N   S   I   K   L   T   S   V   V   F   I   L   I   C   C   F   I   I

CTG GAG AAC ATC TTT GTC TTG CTG ACC ATT TGG AAA ACC AAG AAA TTC CAC CGA CCC ATG TAC TAT TTT
 L   E   N   I   F   V   L   L   T   I   W   K   T   K   K   F   H   R   P   M   Y   Y   F

ATT GGC AAT CTG GCC CTC TCA GAC CTG TTG GCA GGA GTA GCC TAC ACA GCT AAC CTG CTC TTG TCT GGG
 I   G   N   L   A   L   S   D   L   L   A   G   V   A   Y   T   A   N   L   L   L   S   G

GCC ACC ACC TAC AAG CTC ACT CCC GCC CAG TGG TTT CTG CGG GAA GGG AGT ATG TTT GTG GCC CTG TCA
 A   T   T   Y   K   L   T   P   A   Q   W   F   L   R   E   G   S   M   F   V   A   L   S

GCC TCC GTG TTC AGT CTC CTC GCC ATC GCC ATT GAG CGC TAT ATC ACA ATG CTG AAA ATG AAA CTC CAC
 A   S   V   F   S   L   L   A   I   A   I   E   R   Y   I   T   M   L   K   M   K   L   H

AAC GGG AGC AAT AAC TTC CGC CTC TTC CTG CTA ATC AGC GCC TGC TGG GTC ATC TCC CTC ATC CTG GGT
 N   G   S   N   N   F   R   L   F   L   L   I   S   A   C   W   V   I   S   L   I   L   G

GGC CTG CCT ATC ATG GGC TGG AAC TGC ATC AGT GCG CTG TCC AGC TGC TCC ACC GTG CTG CCG CTC TAC
 G   L   P   I   M   G   W   N   C   I   S   A   L   S   S   C   S   T   V   L   P   L   Y

CAC AAG CAC TAT ATC CTC TTC TGC ACC ACG GTC TTC ACT CTG CTT CTG CTC TCC ATC GTC ATT CTG TAC
 H   K   H   Y   I   L   F   C   T   T   V   F   T   L   L   L   L   S   I   V   I   L   Y

TGC AGA ATC TAC TCC TTG GTC AGG ACT CGG AGC CGC CGC CTG ACG TTC CGC AAG AAC ATT TCC AAG GCC
 C   R   I   Y   S   L   V   R   T   R   S   R   R   L   T   F   R   K   N   I   S   K   A

AGC CGC AGC TCT GAG AAG TCG CTG GCG CTG CTC AAG ACC GTA ATT ATC GTC CTG AGC GTC TTC ATC GCC
 S   R   S   S   E   K   S   L   A   L   L   K   T   V   I   I   V   L   S   V   F   I   A

TGC TGG GCA CCG CTC TTC ATC CTG CTC CTG CTG GAT GTG GGC TGC AAG GTG AAG ACC TGT GAC ATC CTC
 C   W   A   P   L   F   I   L   L   L   L   D   V   G   C   K   V   K   T   C   D   I   L

TTC AGA GCG GAG TAC TTC CTG GTG TTA GCT GTG CTC AAC TCC GGC ACC AAC CCC ATC ATT TAC ACT CTG
 F   R   A   E   Y   F   L   V   L   A   V   L   N   S   G   T   N   P   I   I   Y   T   L

ACC AAC AAG GAG ATG CGT CGG GCC TTC ATC CGG ATC ATG TCC TGC TGC AAG TGC CCG AGC GGA GAC TCT
 T   N   K   E   M   R   R   A   F   I   R   I   M   S   C   C   K   C   P   S   G   D   S

GCT GGC AAA TTC AAG CGA CCC ATC ATC GCC GGC ATG GAA TTC AGC CGC AGC AAA TCG GAC AAT TCC TCC
 A   G   K   F   K   R   P   I   I   A   G   M   E   F   S   R   S   K   S   D   N   S   S

CAC CCC CAG AAA GAC GAA GGG GAC AAC CCA GAG ACC ATT ATG TCT TCT GGA AAC GTC AAC TCT TCT TCC
 H   P   Q   K   D   E   G   D   N   P   E   T   I   M   S   S   G   N   V   N   S   S   S

TAG TGA TGA
    Stp Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS176M	ChemiScreen™ S_1P_1 Lysophospholipid receptor membrane prep
HTS176C	ChemiScreen™ Calcium Optimized Cell Line expressing $S1P_1$ Lysophospholipid Receptor

Note: Chem-4 cells are derived from Chem-1 cells.

REFERENCES

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2. Liu, Y., Wada, R., Yamashita, T., Mi, Y., Deng, C. X., Hobson, J. P., Rosenfeldt, H. M., Nava, V. E., Chae, S. S., Lee, M. J., Liu, C. H., Hla, T., Spiegel, S., and Proia, R. L. (2000). Edg-1, the G protein–coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation *J. Clin. Invest.* 106: 951–961
3. Matloubian, M., Lo, C. G., Cinamon, G., Lesneski, M. J., Xu, Y., Brinkmann, V., Allende, M. L., Proia, R. L., and Cyster, J. G. (2004) Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 427: 355–360

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