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PRODUCT DATASHEET

Ready-to-Assay™ S_1P_5 Lysophospholipid Receptor Frozen Cells

CATALOG NUMBER: HTS193RTA

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). S_1P_5 can couple with Gi/o and G12/13, and it mediates S1P induced adenylate cyclase inhibition and Ca^{2+} mobilization like the other S1P receptors. However, unlike the other S1P receptors, it mediates inhibition of MAPK activation and cell proliferation (Im *et al*., 2000). S_1P_5 is predominantly expressed in the white matter tracts and oligodendrocytes and is particularly abundant in the anterior commissure, corpus collosum, and optic tract (Terai *et al*., 2003). S1P induces process retraction in pre-oligodendrocytes and supports cell survival in mature oligodendrocytes by activating S_1P_5, which indicates a role for S_1P_5 in maturation and myelination of oligodendrocytes (Jaillard *et al*., 2005). Cloned human S_1P_5-expressing cell line is made in the Chem-5 host, which supports high levels of recombinant S_1P_5 expression 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_5 Receptor.

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

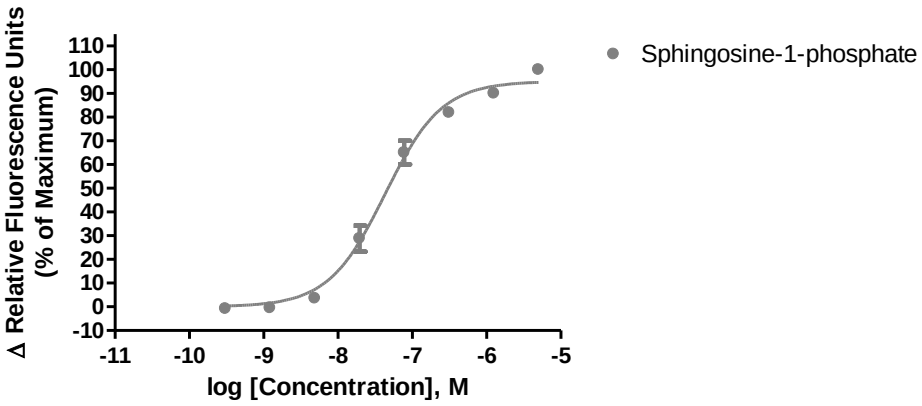

Figure 1. Representative data for activation of S_1P_5 receptor. Calcium flux in S_1P_5 –expressing Chem-5 cell line induced by Sphingosine-1-phosphate. S_1P_5 –expressing Chem-5 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,000 RLU (Relative Light Units).

Table 1. EC_{50} value of S_1P_5 -expressing Chem-5 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Sphingosine-1-phosphate	Calcium Flux	50	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 as well as an exogenous proprietary promiscuous Gα protein.

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EXONGENOUS GENE EXPRESSION

EDG8 cDNA (Accession Number: NM_030760; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GAG TCG GGG CTG CTG CGG CCG GCG CCG GTG AGC
                                                 M   E   S   G   L   L   R   P   A   P   V   S

GAG GTC ATC GTC CTG CAT TAC AAC TAC ACC GGC AAG CTC CGC GGT GCG CGC TAC CAG CCG GGT GCC GGC
 E   V   I   V   L   H   Y   N   Y   T   G   K   L   R   G   A   R   Y   Q   P   G   A   G

CTG CGC GCC GAC GCC GTG GTG TGC CTG GCG GTG TGC GCC TTC ATC GTG CTA GAG AAT CTA GCC GTG TTG
 L   R   A   D   A   V   V   C   L   A   V   C   A   F   I   V   L   E   N   L   A   V   L

TTG GTG CTC GGA CGC CAC CCG CGC TTC CAC GCT CCC ATG TTC CTG CTC CTG GGC AGC CTC ACG TTG TCG
 L   V   L   G   R   H   P   R   F   H   A   P   M   F   L   L   L   G   S   L   T   L   S

GAT CTG CTG GCA GGC GCC GCC TAC GCC GCC AAC ATC CTA CTG TCG GGG CCG CTC ACG CTG AAA CTG TCC
 D   L   L   A   G   A   A   Y   A   A   N   I   L   L   S   G   P   L   T   L   K   L   S

CCC GCG CTC TGG TTC GCA CGG GAG GGA GGC GTC TTC GTG GCA CTC ACT GCG TCC GTG CTG AGC CTC CTG
 P   A   L   W   F   A   R   E   G   G   V   F   V   A   L   T   A   S   V   L   S   L   L

GCC ATC GCG CTG GAG CGC AGC CTC ACC ATG GCG CGC AGG GGG CCC GCG CCC GTC TCC AGT CGG GGG CGC
 A   I   A   L   E   R   S   L   T   M   A   R   R   G   P   A   P   V   S   S   R   G   R

ACG CTG GCG ATG GCA GCC GCG GCC TGG GGC GTG TCG CTG CTC CTC GGG CTC CTG CCA GCG CTG GGC TGG
 T   L   A   M   A   A   A   A   W   G   V   S   L   L   L   G   L   L   P   A   L   G   W

AAT TGC CTG GGT CGC CTG GAC GCT TGC TCC ACT GTC TTG CCG CTC TAC GCC AAG GCC TAC GTG CTC TTC
 N   C   L   G   R   L   D   A   C   S   T   V   L   P   L   Y   A   K   A   Y   V   L   F

TGC GTG CTC GCC TTC GTG GGC ATC CTG GCC GCG ATC TGT GCA CTC TAC GCG CGC ATC TAC TGC CAG GTA
 C   V   L   A   F   V   G   I   L   A   A   I   C   A   L   Y   A   R   I   Y   C   Q   V

CGC GCC AAC GCG CGG CGC CTG CCG GCA CGG CCC GGG ACT GCG GGG ACC ACC TCG ACC CGG GCG CGT CGC
 R   A   N   A   R   R   L   P   A   R   P   G   T   A   G   T   T   S   T   R   A   R   R

AAG CCG CGC TCG CTG GCC TTG CTG CGC ACG CTC AGC GTG GTG CTC CTG GCC TTT GTG GCA TGT TGG GGC
 K   P   R   S   L   A   L   L   R   T   L   S   V   V   L   L   A   F   V   A   C   W   G

CCC CTC TTC CTG CTG CTG TTG CTC GAC GTG GCG TGC CCG GCG CGC ACC TGT CCT GTA CTC CTG CAG GCC
 P   L   F   L   L   L   L   L   D   V   A   C   P   A   R   T   C   P   V   L   L   Q   A

GAT CCC TTC CTG GGA CTG GCC ATG GCC AAC TCA CTT CTG AAC CCC ATC ATC TAC ACG CTC ACC AAC CGC
 D   P   F   L   G   L   A   M   A   N   S   L   L   N   P   I   I   Y   T   L   T   N   R

GAC CTG CGC CAC GCG CTC CTG CGC CTG GTC TGC TGC GGA CGC CAC TCC TGC GGC AGA GAC CCG AGT GGC
 D   L   R   H   A   L   L   R   L   V   C   C   G   R   H   S   C   G   R   D   P   S   G

TCC CAG CAG TCG GCG AGC GCG GCT GAG GCT TCC GGG GGC CTG CGC CGC TGC CTG CCC CCG GGC CTT GAT
 S   Q   Q   S   A   S   A   A   E   A   S   G   G   L   R   R   C   L   P   P   G   L   D

GGG AGC TTC AGC GGC TCG GAG CGC TCA TCG CCC CAG CGC GAC GGG CTG GAC ACC AGC GGC TCC ACA GGC
 G   S   F   S   G   S   E   R   S   S   P   Q   R   D   G   L   D   T   S   G   S   T   G

AGC CCC GGT GCA CCC ACA GCC GCC CGG ACT CTG GTA TCA GAA CCG GCT GCA GAC TGA
 S   P   G   A   P   T   A   A   R   T   L   V   S   E   P   A   A   D  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS193M	ChemiScreen™ S_1P_5 Lysophospholipid Receptor membrane prep

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

REFERENCES

1. Im DS *et al*. (2000) Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. *J. Biol. Chem.*275:14281-6
2. Jaillard C *et al*. (2005) Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *J. Neurosci.* 25: 1459-1469.
3. Spiegel S and Milstien S. (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat. Rev.Mol.CellBiol.*4:397-407.
4. Terai K *et al.* (2003) Edg-8 receptors are preferentially expressed in oligodendrocyte lineage cells of the rat CNS. *Neuroscience* 116: 1053-1062

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