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PRODUCT DATASHEET

ChemiScreen™ $S1P_3$ Lysophospholipid Receptor Stable Cell Line

CATALOG NUMBER: HTS097C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Sphingosine 1-phosphate (S1P) is a bioactive lipid that binds to and activates a family of GPCRs, $S1P_{1-5}$ (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). The cloned human $S1P_3$ -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant $S1P_3$ 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 antagonists of interactions between $S1P_3$ and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay

APPLICATION DATA

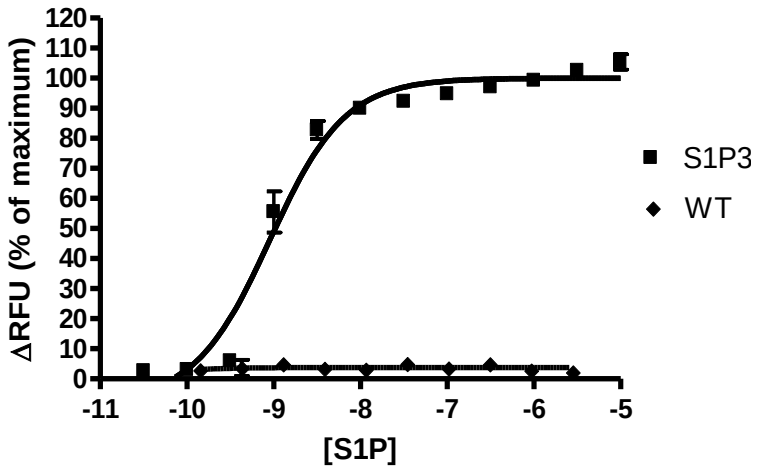

Figure 1. Representative data for activation of the $S1P_3$ receptor stably expressed in Chem-1 cells induced by S1P using a fluorescent calcium flux assay. $S1P_3$–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of $S1P_3$-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
S1P	Calcium Flux - Fluorescence	0.9	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
S1P ligand	Sigma: S9666
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human $S1P_3$ cDNA (Accession Number: NM_005226; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

```
   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-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS097M	ChemiScreen™ $S1P_3$ 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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