eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ sst_3 Somatostatin Receptor Frozen Cells

CATALOG NUMBER: HTS171RTA

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 over night recovery, assays for calcium response.

Somatostatin is a 14 or 28 amino acid regulatory peptide that inhibits hormone secretion from the pituitary, pancreas, and other endocrine sites. A family of 6 GPCRs, sst_1, sst_{2A}, sst_{2B}, sst_3, sst_4 and sst_5, mediate the biological activity of somatostatins. The somatostatin receptors couple to G_i to inhibit cAMP production, and also increase MAP kinase signalling. Several tumors have been shown to overexpress somatostatin receptors, and binding of somatostatin to these tumor cells stimulates or inhibits proliferation, depending on the receptor subtypes expressed (Olias *et al*., 2004). However, sst_3 appears to promote apoptosis, and expression of sst_3 was found to be lower in gastric cancer cells than in normal gastric mucosa, in proportion to susceptibility to apoptosis induced by somatostatin analogs (Sharma *et al*., 1996; Hu *et al*., 2004). In addition, sst_3 is expressed in smooth muscle cells and mediates gastrointestinal contractions. Cloned human sst_3-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant sst_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 sst_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

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

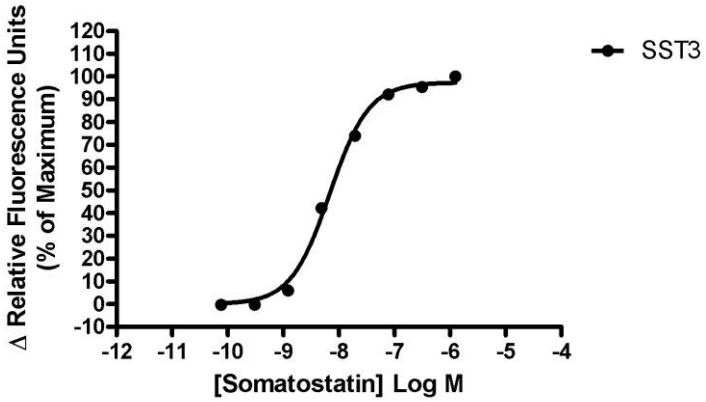

Figure 1. Representative data for activation of sst_3 receptor. Calcium flux in sst_3–expressing Chem-1 cell line induced by Somatostatin. sst_3–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,000 RLU (Relative Light Units).

Table 1. EC_{50} value of sst_3-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Somatostatin	Calcium Flux	6	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	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Somatostatin ligand	Sigma: S9129
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/3574
Black (clear Bottom) cell assay plates	Corning: 3904/3712

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 protein

EXONGENOUS GENE EXPRESSION

SSTR3 cDNA (Accession Number: NM_001051.2; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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   1 - ATG GAC ATG CTT CAT CCA TCA TCG GTG TCC ACG ACC TCA GAA CCT GAG AAT GCC TCC TCG GCC TGG CCC CCA  - 72
   1 -  M   D   M   L   H   P   S   S   V   S   T   T   S   E   P   E   N   A   S   S   A   W   P   P  - 24
  73 - GAT GCC ACC CTG GGC AAC GTG TCG GCA GGC CCA AGC CCG GCA GGG CTG GCC GTC AGT GGC GTT CTG ATC CCC  - 144
  25 -  D   A   T   L   G   N   V   S   A   G   P   S   P   A   G   L   A   V   S   G   V   L   I   P  - 48
 145 - CTG GTC TAC CTG GTG GTG TGC GTG GTG GGC CTG CTG GGT AAC TCG CTG GTC ATC TAT GTG GTC CTG CGG CAC  - 216
  49 -  L   V   Y   L   V   V   C   V   V   G   L   L   G   N   S   L   V   I   Y   V   V   L   R   H  - 72
 217 - ACG GCC AGC CCT TCA GTC ACC AAC GTC TAC ATC CTC AAC CTG GCG CTG GCC GAC GAG CTC TTC ATG CTG GGG  - 288
  73 -  T   A   S   P   S   V   T   N   V   Y   I   L   N   L   A   L   A   D   E   L   F   M   L   G  - 96
 289 - CTG CCC TTC CTG GCC GCC CAG AAC GCC CTG TCC TAC TGG CCC TTC GGC TCC CTC ATG TGC CGC CTG GTC ATG  - 360
  97 -  L   P   F   L   A   A   Q   N   A   L   S   Y   W   P   F   G   S   L   M   C   R   L   V   M  - 120
 361 - GCG GTG GAT GGC ATC AAC CAG TTC ACC AGC ATA TTC TGC CTG ACT GTC ATG AGC GTG GAC CGC TAC CTG GCC  - 432
 121 -  A   V   D   G   I   N   Q   F   T   S   I   F   C   L   T   V   M   S   V   D   R   Y   L   A  - 144
 433 - GTG GTA CAT CCC ACC CGC TCA GCC CGC TGG CGC ACA GCT CCG GTG GCC CGC ACG GTC AGC GCG GCT GTG TGG  - 504
 145 -  V   V   H   P   T   R   S   A   R   W   R   T   A   P   V   A   R   T   V   S   A   A   V   W  - 168
 505 - GTG GCC TCA GCC GTG GTG GTG CTG CCC GTG GTG GTC TTC TCG GGA GTG CCC CGC GGC ATG AGC ACC TGC CAC  - 576
 169 -  V   A   S   A   V   V   V   L   P   V   V   V   F   S   G   V   P   R   G   M   S   T   C   H  - 192
 577 - ATG CAG TGG CCC GAG CCG GCG GCG GCC TGG CGA GCC GGC TTC ATC ATC TAC ACG GCC GCA CTG GGC TTC TTC  - 648
 193 -  M   Q   W   P   E   P   A   A   A   W   R   A   G   F   I   I   Y   T   A   A   L   G   F   F  - 216
 649 - GGG CCG CTG CTG GTC ATC TGC CTC TGC TAC CTG CTC ATC GTG GTG AAG GTG CGC TCA GCT GGG CGC CGG GTG  - 720
 217 -  G   P   L   L   V   I   C   L   C   Y   L   L   I   V   V   K   V   R   S   A   G   R   R   V  - 240
 721 - TGG GCA CCC TCG TGC CAG CGG CGG CGG CGC TCC GAA CGC AGG GTC ACG CGC ATG GTG GTG GCC GTG GTG GCA  - 792
 241 -  W   A   P   S   C   Q   R   R   R   R   S   E   R   R   V   T   R   M   V   V   A   V   V   A  - 264
 793 - CTC TTC GTG CTC TGC TGG ATG CCC TTC TAT GTG CTC AAC ATC GTC AAC GTG GTG TGC CCA CTG CCC GAG GAG  - 864
 265 -  L   F   V   L   C   W   M   P   F   Y   V   L   N   I   V   N   V   V   C   P   L   P   E   E  - 288
 865 - CCT GCC TTC TTT GGG CTC TAC TTC CTG GTG GTG GCG CTG CCC TAT GCC AAC AGC TGT GCC AAC CCC ATC CTT  - 936
 289 -  P   A   F   F   G   L   Y   F   L   V   V   A   L   P   Y   A   N   S   C   A   N   P   I   L  - 312
 937 - TAT GGC TTC CTC TCC TAC CGC TTC AAG CAG GGC TTC CGC AGG GTC CTG CTG CGG CCC TCC CGC CGT GTG CGC -1008
 313 -  Y   G   F   L   S   Y   R   F   K   Q   G   F   R   R   V   L   L   R   P   S   R   R   V   R  - 336
1009 - AGC CAG GAG CCC ACT GTG GGG CCC CCG GAG AAG ACT GAG GAG GAG GAT GAG GAG GAG GAG GAT GGG GAG GAG -1080
 337 -  S   Q   E   P   T   V   G   P   P   E   K   T   E   E   E   D   E   E   E   E   D   G   E   E  - 360
1081 - AGC AGG GAG GGG GGC AAG GGG AAG GAG ATG AAC GGC CGG GTC AGC CAG ATC ACG CAG CCT GGC ACC AGC GGG -1152
 361 -  S   R   E   G   G   K   G   K   E   M   N   G   R   V   S   Q   I   T   Q   P   G   T   S   G  - 384
1153 - CAG GAG CGG CCG CCC AGC AGA GTG GCC AGC AAG GAG CAG CAG CTC CTA CCC CAA GAG GCT TCC ACT GGG GAG -1224
 385 -  Q   E   R   P   P   S   R   V   A   S   K   E   Q   Q   L   L   P   Q   E   A   S   T   G   E  - 408
1225 - AAG TCC AGC ACG ATG CGC ATC AGC TAC CTG TGA
 409 -  K   S   S   T   M   R   I   S   Y   L  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS171M	ChemiScreen™ sst_3 Somatostatin receptor membrane prep

REFERENCES

1. Hu C *et al.* (2004) The effect of somatostatin and SSTR3 on proliferation and apoptosis of gastric cancer cells. *Cancer Biol Ther.* 3(8): 726-730.
2. Olias G *et al.* (2004) Regulation and function of somatostatin receptors. *J. Neurochem.* 89: 1057-1091.
3. Sharma K *et al.* (1996) Subtype-selective induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. *Mol. Endocrinol.* 10: 1688-1696

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