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PRODUCT DATASHEET

Ready-to-Assay™ sst_5 Somatostatin Receptor Frozen Cells

CATALOG NUMBER: HTS139RTA

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.

Somatostatin (sst) is a multifunctional peptide with two biologically active forms, sst-14 and sst-28, which are synthesized in neurons throughout the brain as well as in peripheral tissues such as the pancreas and the gut (Gillies, 1997). SST exerts a diverse array of effects that include inhibition of endocrine secretion, modulation of neurotransmission, and regulation of cell proliferation by stimulating a family of five G-protein-coupled receptors. Somatostatin receptor sst_5 is an inhibitory G protein-coupled receptor that exerts a strong cytostatic effect on various cell types. In mice, sst_5 mediates somatostatin inhibition of pancreatic insulin secretion and contributes to the regulation of glucose homeostasis and insulin sensitivity (Strowski *et al.,* 2003). In addition, deficiency of sst_5 leads to subtype-selective sexually dimorphic changes in the expression of both brain and pancreatic somatostatins (Ramirez *et al*., 2004). Cloned human sst_5-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant sst_5 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_5.

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

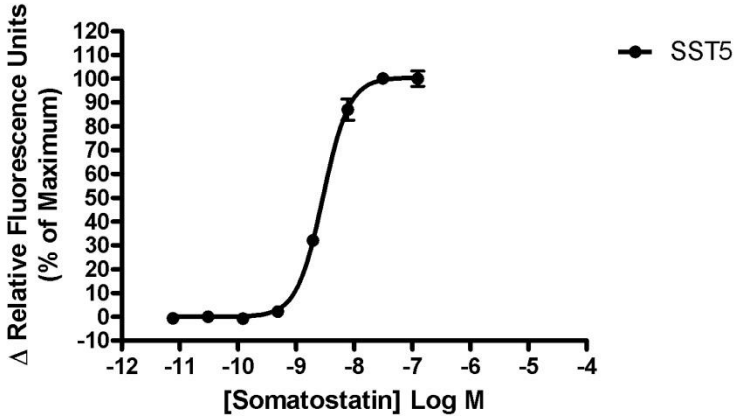

Figure 1. Representative data for activation of sst_5 receptor. Calcium flux in sst_5–expressing Chem-1 cell line induced by Somatostatin. sst_5–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 17,000 RLU (Relative Light Units).

Table 1. EC_{50} values of SST_5-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Somatostatin	Calcium Flux	3	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
Somatostatin ligand	Sigma: S9129
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 Gα15 protein.

EXONGENOUS GENE EXPRESSION

SSTR5 cDNA (Accession Number: NM_001053.1; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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  1 - ATG GAG CCC CTG TTC CCA GCC TCC ACG CCC AGC TGG AAC GCC TCC TCC CCG GGG GCT GCC TCT GGA GGC  - 69
  1 -  M   E   P   L   F   P   A   S   T   P   S   W   N   A   S   S   P   G   A   A   S   G   G  - 23

 70 - GGT GAC AAC AGG ACG CTG GTG GGG CCG GCG CCC TCG GCA GGG GCC CGG GCG GTG CTG GTG CCC GTG CTG  - 138
 24 -  G   D   N   R   T   L   V   G   P   A   P   S   A   G   A   R   A   V   L   V   P   V   L  - 46

139 - TAC CTG CTG GTG TGT GCG GCC GGG CTG GGC GGG AAC ACG CTG GTC ATC TAC GTG GTG CTG CGC TTC GCC  - 207
 47 -  Y   L   L   V   C   A   A   G   L   G   G   N   T   L   V   I   Y   V   V   L   R   F   A  - 69

208 - AAG ATG AAG ACC GTC ACC AAC ATC TAC ATT CTC AAC CTG GCA GTG GCC GAC GTC CTG TAC ATG CTG GGG  - 276
 70 -  K   M   K   T   V   T   N   I   Y   I   L   N   L   A   V   A   D   V   L   Y   M   L   G  - 92

277 - CTG CCT TTC CTG GCC ACG CAG AAC GCC GCG TCC TTC TGG CCC TTC GGC CCC GTC CTG TGC CGC CTG GTC  - 345
 93 -  L   P   F   L   A   T   Q   N   A   A   S   F   W   P   F   G   P   V   L   C   R   L   V  - 115

346 - ATG ACG CTG GAC GGC GTC AAC CAG TTC ACC AGT GTC TTC TGC CTG ACA GTC ATG AGC GTG GAC CGC TAC  - 414
116 -  M   T   L   D   G   V   N   Q   F   T   S   V   F   C   L   T   V   M   S   V   D   R   Y  - 138

415 - CTG GCA GTG GTG CAC CCG CTG AGC TCG GCC CGC TGG CGC CGC CCG CGT GTG GCC AAG CTG GCG AGC GCC  - 483
139 -  L   A   V   V   H   P   L   S   S   A   R   W   R   R   P   R   V   A   K   L   A   S   A  - 161

484 - GCG GCC TGG GTC CTG TCT CTG TGC ATG TCG CTG CCG CTC CTG GTG TTC GCG GAC GTG CAG GAG GGC GGT  - 552
162 -  A   A   W   V   L   S   L   C   M   S   L   P   L   L   V   F   A   D   V   Q   E   G   G  - 184

553 - ACC TGC AAC GCC AGC TGG CCG GAG CCC GTG GGG CTG TGG GGC GCC GTC TTC ATC ATC TAC ACG GCC GTG  - 621
185 -  T   C   N   A   S   W   P   E   P   V   G   L   W   G   A   V   F   I   I   Y   T   A   V  - 207

622 - CTG GGC TTC TTC GCG CCG CTG CTG GTC ATC TGC CTG TGC TAC CTG CTC ATC GTG GTG AAG GTG AGG GCG  - 690
208 -  L   G   F   F   A   P   L   L   V   I   C   L   C   Y   L   L   I   V   V   K   V   R   A  - 230

691 - GCG GGC GTG CGC GTG GGC TGC GTG CGG CGG CGC TCG GAG CGG AAG GTG ACG CGC ATG GTG TTG GTG GTG  - 759
231 -  A   G   V   R   V   G   C   V   R   R   R   S   E   R   K   V   T   R   M   V   L   V   V  - 253

760 - GTG CTG GTG TTT GCG GGA TGT TGG CTG CCC TTC TTC ACC GTC AAC ATC GTC AAC CTG GCC GTG GCG CTG  - 828
254 -  V   L   V   F   A   G   C   W   L   P   F   F   T   V   N   I   V   N   L   A   V   A   L  - 276

829 - CCC CAG GAG CCC GCC TCC GCC GGC CTC TAC TTC TTC GTG GTC ATC CTC TCC TAC GCC AAC AGC TGT GCC  - 897
277 -  P   Q   E   P   A   S   A   G   L   Y   F   F   V   V   I   L   S   Y   A   N   S   C   A  - 299

898 - AAC CCC GTC CTC TAC GGC TTC CTC TCT GAC AAC TTC CGC CAG AGC TTC CAG AAG GTT CTG TGC CTC CGC  - 966
300 -  N   P   V   L   Y   G   F   L   S   D   N   F   R   Q   S   F   Q   K   V   L   C   L   R  - 322

967 - AAG GGC TCT GGT GCC AAG GAC GCT GAC GCC ACG GAG CTG CGT CCA GAC AGG ATC CGG CAG CAG CAG GAG  - 1035
323 -  K   G   S   G   A   K   D   A   D   A   T   E   L   R   P   D   R   I   R   Q   Q   Q   E  - 345

1036 - GCC ACG CCG CCC GCG CAC CGC GCC GCA GCC AAC GGG CTT ATG CAG ACC AGC AAG CTG TGA
 346 -  A   T   P   P   A   H   R   A   A   A   N   G   L   M   Q   T   S   K   L  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS139M	ChemiScreen™ sst_5 Somatostatin receptor membrane prep

REFERENCES

1. Gillies G (1997) Somatostatin: the neuroendocrine story. *Trends Pharmacol. Sci.* 18: 87-95.
2. Strowski, MZ, Kohler, M., Chen, HY *et al.* (2003). Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. *Mol. Endocrinol.* 17: 93–106.
3. Ramirez, JL., Grant, M., Norman, M. *et al.* (2004) Deficiency of somatostatin (SST) receptor type 5 (SSTR5) is associated with sexually dimorphic changes in the expression of SST and SST receptors in brain and pancreas. *Mol. Cell. Endocrinol.* 221: 105–119.

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