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PRODUCT DATASHEET

Ready-to-Assay™ sst_2 Somatostatin Receptor Frozen Cells

CATALOG NUMBER: HTS028RTA

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 (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_2 mRNA is predominantly expressed in central nervous system. Study using sst_2 knock-out mice has found the increased anxiety-related behaviour while locomotor and exploratory activity was decreased in stress-inducing situations (coupled with an increase in pituitary ACTH release, a regulator of the stress response) (Viollet *et al.*, 2000). In the periphery, inhibition of glucagon release by sst in mouse islets is primarily mediated via sst_2 (Strowski *et al.*, 2000). In addition, endogenous sst functions through sst_2 to suppress gastric acid secretion through inhibition of gastrin activity (Martinez *et al.*, 1998). Cloned human sst_2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant sst_2 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_2.

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

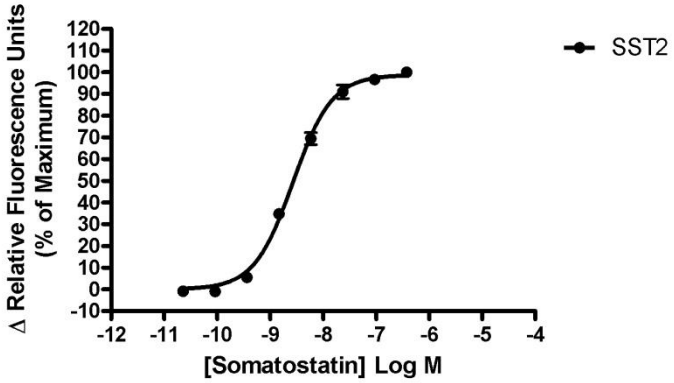

Figure 1. Representative data for activation of sst_2 receptor. Calcium flux in sst_2–expressing Chem-1 cell line induced by Somatostatin. sst_2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 5,000 RLU (Relative Light Units).

Table 1. EC_{50} value of sst_2-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

SSTR2 cDNA (Accession Number: NM_001050.2; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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   1 - ATG GAC ATG GCG GAT GAG CCA CTC AAT GGA AGC CAC ACA TGG CTA TCC ATT CCA TTT GAC CTC AAT GGC TCT  - 72
   1 -  M   D   M   A   D   E   P   L   N   G   S   H   T   W   L   S   I   P   F   D   L   N   G   S  - 24

  73 - GTG GTG TCA ACC AAC ACC TCA AAC CAG ACA GAG CCG TAC TAT GAC CTG ACA AGC AAT GCA GTC CTC ACA TTC  - 144
  25 -  V   V   S   T   N   T   S   N   Q   T   E   P   Y   Y   D   L   T   S   N   A   V   L   T   F  - 48

 145 - ATC TAT TTT GTG GTC TGC ATC ATT GGG TTG TGT GGC AAC ACA CTT GTC ATT TAT GTC ATC CTC CGC TAT GCC  - 216
  49 -  I   Y   F   V   V   C   I   I   G   L   C   G   N   T   L   V   I   Y   V   I   L   R   Y   A  - 72

 217 - AAG ATG AAG ACC ATC ACC AAC ATT TAC ATC CTC AAC CTG GCC ATC GCA GAT GAG CTC TTC ATG CTG GGT CTG  - 288
  73 -  K   M   K   T   I   T   N   I   Y   I   L   N   L   A   I   A   D   E   L   F   M   L   G   L  - 96

 289 - CCT TTC TTG GCT ATG CAG GTG GCT CTG GTC CAC TGG CCC TTT GGC AAG GCC ATT TGC CGG GTG GTC ATG ACT  - 360
  97 -  P   F   L   A   M   Q   V   A   L   V   H   W   P   F   G   K   A   I   C   R   V   V   M   T  - 120

 361 - GTG GAT GGC ATC AAT CAG TTC ACC AGC ATC TTC TGC CTG ACA GTC ATG AGC ATC GAC CGA TAC CTG GCT GTG  - 432
 121 -  V   D   G   I   N   Q   F   T   S   I   F   C   L   T   V   M   S   I   D   R   Y   L   A   V  - 144

 433 - GTC CAC CCC ATC AAG TCG GCC AAG TGG AGG AGA CCC CGG ACG GCC AAG ATG ATC ACC ATG GCT GTG TGG GGA  - 504
 145 -  V   H   P   I   K   S   A   K   W   R   R   P   R   T   A   K   M   I   T   M   A   V   W   G  - 168

 505 - GTC TCT CTG CTG GTC ATC TTG CCC ATC ATG ATA TAT GCT GGG CTC CGG AGC AAC CAG TGG GGG AGA AGC AGC  - 576
 169 -  V   S   L   L   V   I   L   P   I   M   I   Y   A   G   L   R   S   N   Q   W   G   R   S   S  - 192

 577 - TGC ACC ATC AAC TGG CCA GGT GAA TCT GGG GCT TGG TAC ACA GGG TTC ATC ATC TAC ACT TTC ATT CTG GGG  - 648
 193 -  C   T   I   N   W   P   G   E   S   G   A   W   Y   T   G   F   I   I   Y   T   F   I   L   G  - 216

 649 - TTC CTG GTA CCC CTC ACC ATC ATC TGT CTT TGC TAC CTG TTC ATT ATC ATC AAG GTG AAG TCC TCT GGA ATC  - 720
 217 -  F   L   V   P   L   T   I   I   C   L   C   Y   L   F   I   I   I   K   V   K   S   S   G   I  - 240

 721 - CGA GTG GGC TCC TCT AAG AGG AAG AAG TCT GAG AAG AAG GTC ACC CGA ATG GTG TCC ATC GTG GTG GCT GTC  - 792
 241 -  R   V   G   S   S   K   R   K   K   S   E   K   K   V   T   R   M   V   S   I   V   V   A   V  - 264

 793 -  TTC ATC TTC TGC TGG CTT CCC TTC TAC ATA TTC AAC GTT TCT TCC GTC TCC ATT GCC ATC AGC CCC ACC CCA  - 864
 265 -  F   I   F   C   W   L   P   F   Y   I   F   N   V   S   S   V   S   I   A   I   S   P   T   P  - 288

 865 - GCC CTT AAA GGC ATG TTT GAC TTT GTG GTG GTC CTC ACC TAT GCT AAC AGC TGT GCC AAC CCT ATC CTA TAT  - 936
 289 -  A   L   K   G   M   F   D   F   V   V   V   L   T   Y   A   N   S   C   A   N   P   I   L   Y  - 312

 937 - GCC TTC TTG TCT GAC AAC TTC AAG AAG AGC TTC CAG AAT GTC CTC TGC TTG GTC AAG GTG AGC GGC ACA GAT  - 1008
 313 -  A   F   L   S   D   N   F   K   K   S   F   Q   N   V   L   C   L   V   K   V   S   G   T   D  - 336

1009 - GAT GGG GAG CGG AGT GAC AGT AAG CAG GAC AAA TCC CGG CTG AAT GAG ACC ACG GAG ACC CAG AGG ACC CTC  - 1080
 337 -  D   G   E   R   S   D   S   K   Q   D   K   S   R   L   N   E   T   T   E   T   Q   R   T   L  - 360

1081 - CTC AAT GGA GAC CTC CAA ACC AGT ATC TGA
 361 -  L   N   G   D   L   Q   T   S   I  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS028M	ChemiScreen™ sst_2 Somatostatin receptor membrane prep

REFERENCES

1. Gillies G (1997) Somatostatin: the neuroendocrine story. *Trends Pharmacol. Sci.* 18: 87-95.
2. Martinez V *et al.* (1998) High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. *Gastroenterology* 114: 1125 - 1132.
3. Strowski MZ *et al.* (2000) Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology* 141: 111 – 117
4. Viollet C *et al.* (2000) Involvement of sst_2 somatostatin receptor in locomotor, exploratory activity and emotional reactivity in mice *Eur. J. Neurosci*. 12: 3761 - 3770.

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