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PRODUCT DATASHEET

ChemiScreen™ SST_5 Somatostatin Receptor Stable Cell Line

CATALOG NUMBER: HTS139C

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.

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). The 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 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between SST_5 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

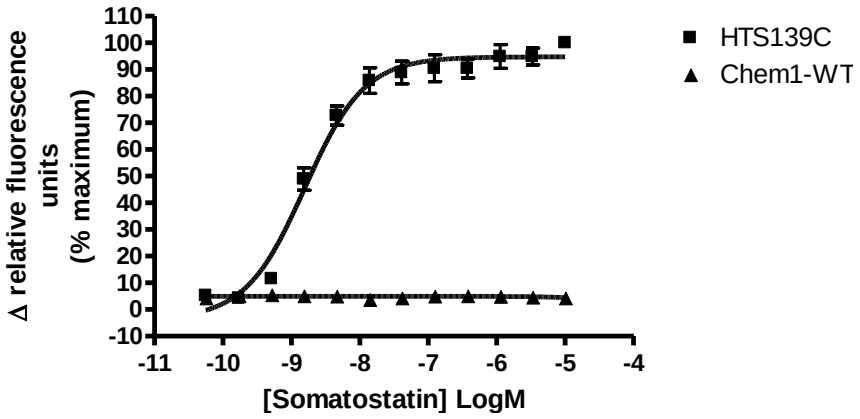

Figure 1. Representative data for activation of the SST_5 receptor stably expressed in Chem-1 cells induced by Somatostatin using a fluorescent calcium flux assay. SST_5–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with 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 7,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Somatostatin	Calcium Flux - Fluorescence	1.6	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
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
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.

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HOST CELL

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

EXOGENOUS GENE EXPRESSION

Human SST_5 cDNA (Accession Number: NM_001053.1; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

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-1	ChemiScreen™ Chem-1 Parental Cell Line (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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