

## PRODUCT DATASHEET

### Ready-to-Assay™ sst<sub>4</sub> Somatostatin Receptor Frozen Cells

#### CATALOG NUMBER: HTS125RTA

**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<sub>2</sub>. Media Component at 4°C (-20°C 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 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<sub>1</sub>, sst<sub>2A</sub>, sst<sub>2B</sub>, sst<sub>3</sub>, sst<sub>4</sub> and sst<sub>5</sub>, mediate the biological activity of somatostatins. The somatostatin receptors couple to G<sub>i</sub> 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). Somatostatin has been implicated in seizure susceptibility in animal models, and activation of sst<sub>4</sub> with selective agonists increases seizure activity (Moneta *et al.*, 2002). Cloned human sst<sub>4</sub>-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant sst<sub>4</sub> 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<sub>4</sub>.

#### 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.

## APPLICATIONS

Calcium Flux Assays

### APPLICATION DATA

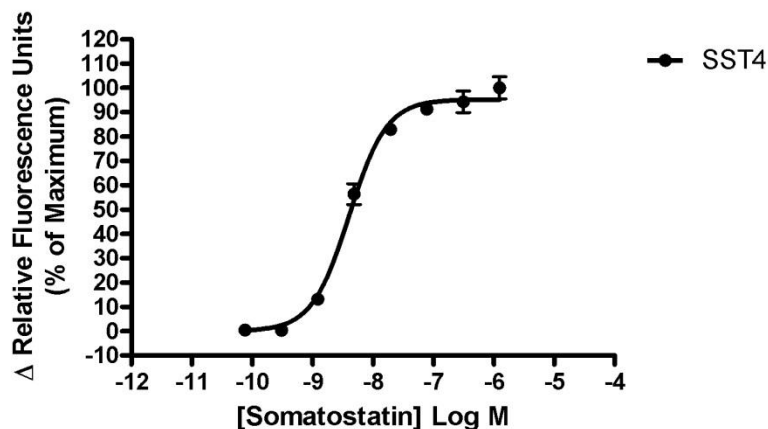


Figure 1. Representative data for activation of sst<sub>4</sub> receptor. Calcium flux in sst<sub>4</sub>-expressing Chem-1 cell line induced by Somatostatin. Sst<sub>4</sub>-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR<sup>TETRA</sup>. Maximal fluorescence signal obtained in this experiment was 3,500 RLU (Relative Light Units).

Table 1. EC<sub>50</sub> values of sst<sub>4</sub>-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Somatostatin	Calcium Flux	4	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 37°C 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 37°C 5% CO<sub>2</sub> 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<sup>2+</sup> dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca<sup>2+</sup> dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca<sup>2+</sup> 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<sup>TETRA</sup>) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR<sup>TETRA</sup>) or emission filter for Ca<sup>2+</sup> 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
Probenecid	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<sup>TETRA</sup>® 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

SSTR4 cDNA (Accession Number: NM\_001052; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

**CODING SEQUENCE**

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ATG AGC GCC CCC TCG ACG CTG CCC CCC GGG GGC GAG
M S A P S T L P P G G E
GAA GGG CTG GGG ACG GCC TGG CCC TCT GCA GCC AAT GCC AGT AGC GCT CCG GCG GAG GCG GAG GAG GCG
E G L G T A W P S A A N A S S A P A E A E E A
GTG GCG GGG CCC GGG GAC GCG CGG GCG GCG GGC ATG GTC GCT ATC CAG TGC ATC TAC GCG CTG GTG TGC
V A G P G D A R A A G M V A I Q C I Y A L V C
CTG GTG GGG CTG GTG GGC AAC GCC CTG GTC ATC TTC GTG ATC CTT CGC TAC GCC AAG ATG AAG ACG GCT
L V G L V G N A L V I F V I L R Y A K M K T A
ACC AAC ATC TAC CTG CTC AAC CTG GCC GTA GCC GAC GAG CTC TTC ATG CTG AGC GTG CCC TTC GTG GCC
T N I Y L L N L A V A D E L F M L S V P F V A
TCG TCG GCC GCC CTG CGC CAC TGG CCC TTC GGC TCC GTG CTG TGC CGC GCG GTG CTC AGC GTC GAC GGC
S S A A L R H W P F G S V L C R A V L S V D G
CTC AAC ATG TTC ACC AGC GTC TTC TGT CTC ACC GTG CTC AGC GTG GAC CGC TAC GTG GCC GTG GTG CAC
L N M F T S V F C L T V L S V D R Y V A V V H
CCT CTG CGC GCG GCG ACC TAC CGG CGG CCC AGC GTG GCC AAG CTC ATC AAC CTG GGC GTG TGG CTG GCA
P L R A A T Y R R P S V A K L I N L G V W L A
TCC CTG TTG GTC ACT CTC CCC ATC GCC ATC TTC GCA GAC ACC AGA CCG GCT CGC GGC GGC CAG GCC GTG
S L L V T L P I A I F A D T R P A R G G Q A V
GCC TGC AAC CTG CAG TGG CCA CAC CCG GCC TGG TCG GCA GTC TTC GTG GTC TAC ACT TTC CTG CTG GGC
A C N L Q W P H P A W S A V F V V Y T F L L G
TTC CTG CTG CCC GTG CTG GCC ATT GGC CTG TGC TAC CTG CTC ATC GTG GGC AAG ATG CGC GCC GTG GCC
F L L P V L A I G L C Y L L I V G K M R A V A
CTG CGC GCT GGC TGG CAG CAG CGC AGG CGC TCG GAG AAG AAA ATC ACC AGG CTG GTG CTG ATG GTC GTG
L R A G W Q Q R R R S E K K I T R L V L M V V
GTC GTC TTT GTG CTC TGC TGG ATG CCT TTC TAC GTG GTG CAG CTG CTG AAC CTC GTC GTG ACC AGC CTT
V V F V L C W M P F Y V V Q L L N L V V T S L
GAT GCC ACC GTC AAC CAC GTG TCC CTT ATC CTC AGC TAT GCC AAC AGC TGC GCC AAC CCT ATT CTC TAT
D A T V N H V S L I L S Y A N S C A N P I L Y
GGC TTC CTC TCC GAC AAC TTC CGC CGA TCC TTC CAG CGG GTT CTC TGC CTG CGC TGC TGC CTC CTG GAA

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G F L S D N F R R S F Q R V L C L R C C L L E  
 GGT GCT GGA GGT GCT GAG GAG GAG CCC CTG GAC TAC TAT GCC ACT GCT CTC AAG AGC AAA GGT GGG GCA  
 G A G G A E E E P L D Y Y A T A L K S K G G A  
 GGG TGC ATG TGC CCC CCA CTC CCC TGC CAG CAG GAA GCC CTG CAA CCA GAA CCC GGC CGC AAG CGC ATC  
 G C M C P P L P C Q Q E A L Q P E P G R K R I  
 CCC CTC ACC AGG ACC ACC ACC TTC TGA  
 P L T R T T T F Stp

## RELATED PRODUCTS

### PRODUCT NUMBER

### DESCRIPTION

**HTSCHEM-1RTA**

Ready-to-Assay™ Chem-1 host frozen cells (control cells)

**HTS125M**

 ChemiScreen™ sst<sub>4</sub> Somatostatin receptor membrane prep

## REFERENCES

1. Moneta D *et al.* (2002) Somatostatin receptor subtypes 2 and 4 affect seizure susceptibility and hippocampal excitatory neurotransmission in mice. *Eur. J. Neurosci.* 16: 843-9.
2. Olias G *et al.* (2004) Regulation and function of somatostatin receptors. *J. Neurochem.* 89: 1057-1091.

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