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ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT sst_2 RECEPTOR

CATALOG NUMBER:	HTS028C	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:	R0710E0015	**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND:

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). Millipore's 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 optimal 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_2 and its ligands.

APPLICATIONS:

Calcium flux assay, ligand binding assays

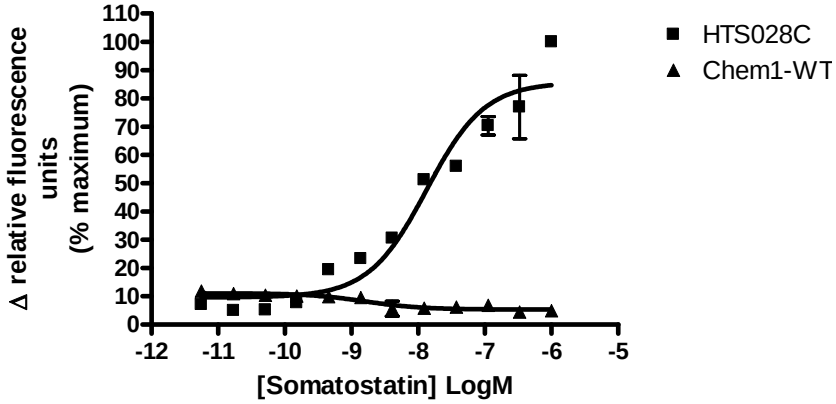

Figure 1. Calcium flux in sst_2–expressing Chem-1 cell line induced by somatostatin. sst_2 expressing Chem-1 cells and Wild-Type Chem-1 cells were loaded with a calcium dye and calcium flux in response to somatostatin (10^{-6} to $10^{-11.25}$ M) was determined in triplicate on a Molecular Devices FLIPR TETRA.

SPECIFICATIONS: EC50 for calcium mobilization by somatostatin: ~13.4nM

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

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TRANSFECTION: Full-length human SSTR2 cDNA encoding sst_2 (Accession Number: NM_001050.2)

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in 90% fetal bovine serum/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
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. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37ºC with 5% CO_2.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca^{++} and Mg^{++} (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37ºC with 5% CO_2 until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5 x 10^6 cells/mL in Chem-1 Freezing Media (cell densities of 2-10 x 10^6 are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at –70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Chem-1 Plating Media for plating for calcium assay.

MEDIA:

Chem-1 Growth Media*:*
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250µg/mL Genetecin/G-418

Chem-1 Plating Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

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Chem-1 Freezing Media:
90% heat-inactivated FBS
10% DMSO (cell culture grade)

REFERENCES:

Gillies G (1997) Somatostatin: the neuroendocrine story. *Trends Pharmacol. Sci.* 18: 87-95.

Martinez V *et al.* (1998) High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. *Gastroenterology* 114: 1125 - 1132.

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.

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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