

Human Recombinant SST4 Somatostatin Receptor Stable Cell Line**Cat. No. M00334****Version 06092014**

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I. INTRODUCTION

Catalog Number: M00334

Cell Line Name: CHO-K1/SST4/Gα15

Gene Synonyms: SSTR4

Expressed Gene: Genbank Accession Number NM_001052; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for SST4 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 400 µg/ml G418, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Somatostatin acts at many sites to inhibit the release of many hormones and other secretory proteins. The biologic effects of somatostatin are probably mediated by a family of G protein-coupled receptors that are expressed in a tissue-specific manner. SST4 is a member of the superfamily of receptors having seven transmembrane segments and is expressed in highest levels in fetal and adult brain and lung. SST4 enhances AMPA receptor-mediated currents in the hippocampus, and interacts with the SST2 receptor.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritidis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

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III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Somatostatin-14 in CHO-K1/SST4/G α 15 and CHO-K1/G α 15 cells

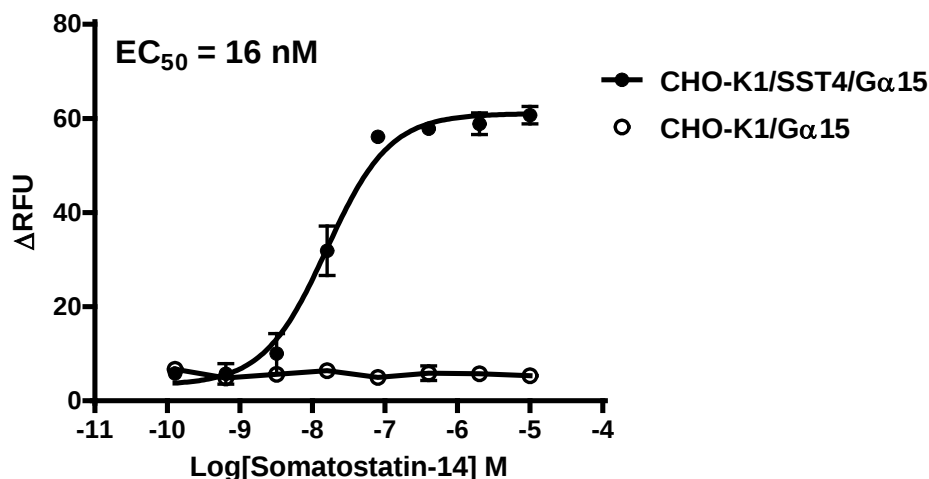


Figure 1. Somatostatin-14-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/SST4/G α 15 and CHO-K1/G α 15 cells. The cells were loaded with Calcium-4 prior to stimulation with an SST14 receptor agonist, Somatostatin-14. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Somatostatin-14 (Mean $\pm$ SD, n = 2). The EC₅₀ of Somatostatin-14 on SST4 co-expressing with G α 15 in CHO-K1 cells was 16 nM. The S/B of Somatostatin-14 on SST4 co-expressing with G α 15 in CHO-K1 cells was 18.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})$$

X is the logarithm of concentration. Y is the response
Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.

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6. Grow the cells in incubator with 37°C, 5 %CO₂.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Rohrer L, Raulf F, Bruns C, *et al.* (1993) Cloning and characterization of a fourth human somatostatin receptor. *Proc Natl Acad Sci USA* 90(9): 4196–200.
2. Moneta, D., Richichi, C., Aliprandi, *et. al.* (2002) Somatostatin receptor subtypes 2 and 4 affect seizure susceptibility and hippocampal excitatory neurotransmission in mice. *Eur J Neurosci.* 16:843-849.

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