

## Human Recombinant SST3 Somatostatin Receptor Stable Cell Line

Cat. No. M00285

Version 07282020

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### I. INTRODUCTION

Catalog Number: M00285

Cell Line Name: CHO-K1/SST3/Gα15

Gene Synonyms: SSTR3, SRIF1c

Expressed gene: Genbank Accession Number NM\_001051; no expressed tags

Host cell: CHO-K1/Gα15

Culture Properties: Adherent

Quantity: 2 vial (>1×10<sup>6</sup> per vial) frozen cells

Stability: More than 16 passages

Application: Functional assay for SST3 receptor

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen), 200 µg/ml Zeocin (Cat. #R250-01, Life Technologies)

Mycoplasma Status: Negative\*

Storage: Liquid nitrogen immediately upon receipt

### II. BACKGROUND

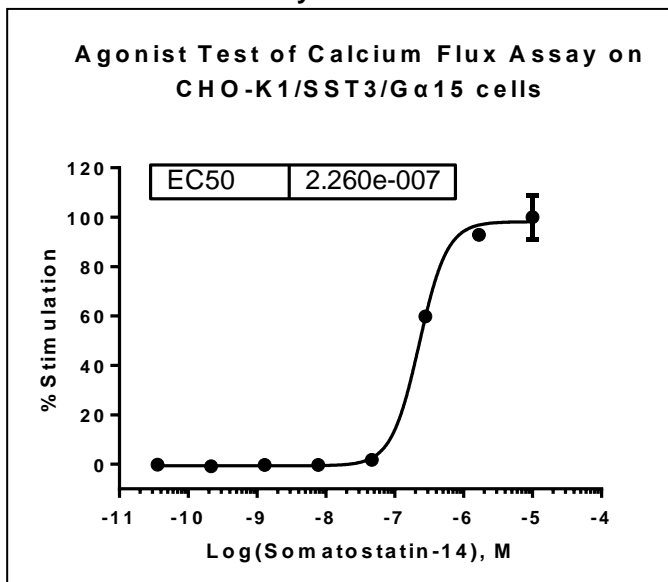
Somatostatin receptors (SSTRs), a family of seven transmembrane (TM) domain G-protein-coupled receptors having five distinct subtypes (termed SSTR1–5), are activated by somatostatin secreted from the nerve and endocrine cells. SSTRs are widely expressed in many tissues, frequently as multiple subtypes that coexist in the same cell. With expressions in a tissue-specific manner, SSTRs are involved in the regulation of secretion of insulin, glucagon and growth hormone as well as cell growth induced by neuronal excitation in both the central and peripheral nervous systems. The five receptors share common signaling pathways such as the inhibition of adenylyl cyclase, activation of phosphotyrosine phosphatase (PTP), and modulation of mitogen-activated protein kinase (MAPK) through G-protein-dependent mechanisms. Aberrant expression of somatostatin receptors is known to be involved in a large number of human tumors.

\* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

The human medullary thyroid carcinoma cell line TT expresses all SSTR subtypes. SSTR3 mRNA is detected in the brain and pancreatic islets. SSTR3 uniquely triggers PTP-dependent apoptosis accompanied by the activation of p53 and pro-apoptotic protein Bax, and displays acute desensitization of adenylyl cyclase coupling.

### III. REPRESENTATIVE DATA

#### Intracellular calcium mobilization assay:



**Figure 1.** Somatostatin-14-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/SST3/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist somatostatin-14. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of Somatostatin-14 (Mean ± SD, n = 2). The EC<sub>50</sub> of Somatostatin-14 on this cell was 0.226 μM.

#### Notes:

- EC<sub>50</sub> value is calculated with four parameter logistic equation:  

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

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.

4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO<sub>2</sub>.
7. Add antibiotic in the following day.

#### **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 %CO<sub>2</sub>.

Subcultivation Ratio: 1:3 to 1:8.

Medium Renewal: Every 2 to 3 days

#### **V. REFERENCES**

1. Yogesh C. Patel (1999) Somatostatin and its receptor family. *Frontiers in Neuroendocrinology* 20(3): 157-198
2. Carruthers A.M., Warner A.J., Michel.A.D., Feniuk W., Humphrey P.P.A. (1999) Activation of adenylate cyclase by human recombinant sst5 receptors expressed in CHO-K1 cells and involvement of Gas proteins. *Br. J. Pharmacol.* 126: 1221-1229
3. Forbes Alderton, Tai-Ping D Fan, and Patrick P A Humphrey (2001) Somatostatin receptor-mediated arachidonic acid mobilization: evidence for partial agonism of synthetic peptides. *Br. J. Pharmacol.* 132(3): 760-766

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