Human Recombinant SST2 Somatostatin Receptor Stable Cell Line

Cat. No. M00325 **Version 07292020**

I. INTRODUCTION

Catalog Number: M00325
Cell Line Name: CHO-K1/SST2/Gα15
Gene Synonyms: SSTR2
Expressed Gene: Genbank Accession Number NM_001050; no expressed tags
Host Cell: CHO-K1/Gα15
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: 16 passages
Application: Functional assay for SST2 receptor (Calcium flux assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Culture Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 400 μg/ml Zeocin (Cat. #R250-01, Life Technologies), 200 μg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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. SST2 is a member of the superfamily of receptors having seven transmembrane segments and is expressed in highest levels in cerebrum and kidney. Studies showed the involvement of the SST2 receptor in the inhibition of glucagon secretion.

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

III. REPRESENTATIVE DATA

Calcium mobilization assay:

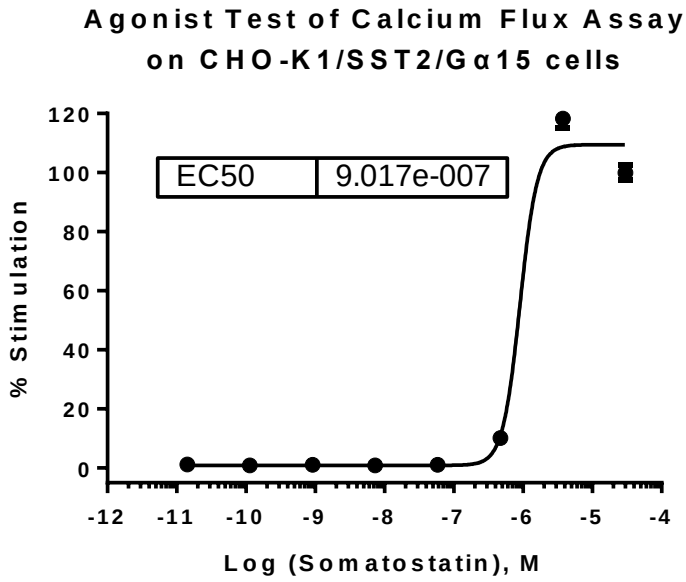

Figure 1. Somatostatin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/SST2/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist somatostatin. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (10-fold dilution) of Somatostatin (Mean ± SD, n = 2). The EC_{50} of somatostatin on this cell was 0.9 µM.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*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.

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. 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.
3. 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_2.

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_2.

Subcultivation Ratio: 1:3 to 1:8
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Yamada Y, Stoffel M, Espinosa R 3rd, *et al.* (1993) Human somatostatin receptor genes: localization to human chromosomes 14, 17, and 22 and identification of simple tandem repeat polymorphisms. *Genomics* 15 (2): 449–52.
2. Zitzer, H; Hönck H H, Bächner D, *et al*. (1999) Somatostatin receptor interacting protein defines a novel family of multidomain proteins present in human and rodent brain. *J. Biol. Chem*. 274 (46): 32997–3001.

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