Human Recombinant KiSS1-derived Peptide Receptor Stable Cell Line

Cat. No. NA **Version 06112020**

I. INTRODUCTION

Catalog Number: M00190
Cell Line Name: CHO-K1/EDG2
Gene Synonyms: KISS1R; AXOR12; GPR54; HOT7T175
Expressed Gene: Genbank Accession Number NM_032551; no expressed tags
Host Cell: CHO-K1
Culture Properties: Adherent
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for KiSS1 receptor
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F12 (Cat. #11765, Life Technologies), 10% FBS
Culture Medium: Ham's F12, 10% FBS, 400 μg/ml G418 (Cat. #10131-035, Gibco)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The KiSS1-derived peptide receptor (also known as GPR54 or the Kisspeptin receptor) is a G_q-coupled receptor which binds the peptide hormone kisspeptin (metastin). The KiSS1 gene inhibits metastatic activity of melanoma and other tumor cell lines, and clinical evidence supports a role for KiSS1 in inhibition of metastasis in human cancer. Kisspeptins and GPR54 also play a central role in hypothalamic regulation of puberty, by directly governing the release of gonadotropin-releasing hormone from the hypothalamus. In addition, mutations in GPR54 in mice and humans result in hypogonadotropic hypogonadism.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by LPA in CHO-K1/EDG2

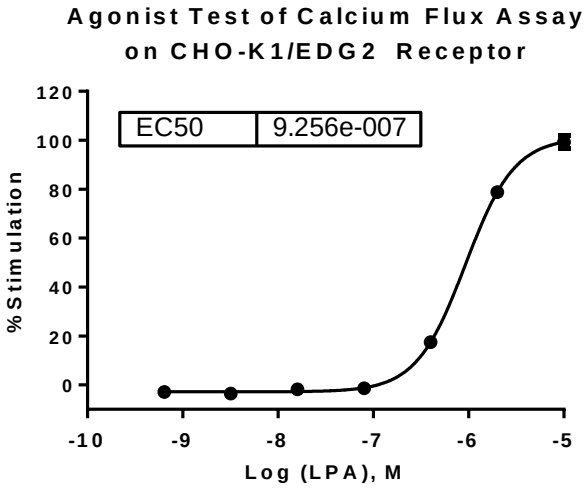

Figure 1: LPA-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/EDG2 cells. The cells were loaded with Calcium-4 prior to stimulation with EDG2 receptor agonist, LPA. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of LPA (Mean ± SD, n = 2). The EC_{50} of LPA on EDG2/CHO-K1 cells was 0.93 μM. The S/B of LPA on EDG2 in CHO-K1 cells was 31.

Note:

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 and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to Background Ratio (S/B) = Top/Bottom

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 for 5 min, and remove the medium.
4. Resuspend the cells with 1 ml complete growth medium.
5. Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
6. Transfer the dish into an incubator of 37°C, 5% CO_2.

7. Add antibiotic into the medium on the next 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 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 during incubation. 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 complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 X g for 5min, and remove the medium.
6. Resuspend the cells in culture medium and transfer the cells to a new culture dish.
7. Transfer the dish into an incubator of 37°C, 5% CO_2.

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

V. REFERENCES

1. Lee DK, Nguyen T, O'Neill GP, et al., Discovery of a receptor related to the galanin receptors (1999). *FEBS Lett*. 446 (1): 103–7.
2. Kotani M, Detheux M, Vandenbogaerde A, et al. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54 (2001). *J. Biol. Chem*. 276 (37): 34631–6.
3. Shahab M, Mastronardi C, Seminara SB, et al. (2005) Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proceedings of the National Academy of Sciences of the United States of America*. 102 (6): 2129–34.
4. Colledge WH (2005) GPR54 and puberty. *Trends Endocrinol. Metab*. 15 (9): 448–53.

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