

Human Recombinant KiSS1-derived Peptide Receptor Stable Cell Line

Cat. No. M00190

Version 07272020

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I. INTRODUCTION

Catalog Number: M00190

Cell Line Name: CHO-K1/KiSS1

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 (>1×10⁶ per vial)

Stability: More than 16 passages

Application: Functional assay for KiSS1 receptor (Calcium flux assay)

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, 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 Kisspeptin-10 in CHO-K1/KiSS1 cells.

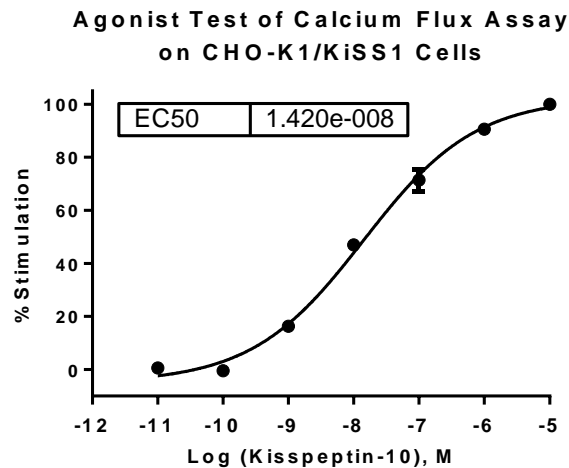


Figure 1: Kisspeptin-10-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/KiSS1 cells. The cells were loaded with Calcium-4 prior to being stimulated with KiSS1 receptor agonist, Kisspeptin-10. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of LPA (Mean \pm SD, n = 2). The EC₅₀ of LPA on CHO-K1/KiSS1 cells was 14.2 nM.

Note:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$
 X is the logarithm of concentration. Y is the response and starts at Bottom and goes to Top with a sigmoid shape.

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 for 5 min, and remove the medium.
- Resuspend the cells with 1 ml complete growth medium.
- Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
- Transfer the dish into an incubator of 37°C, 5% CO₂.
- 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% CO₂.

Subcultivation Ratio: 1:3 to 1:8

Medium Renewal: Every 2 to 3 days

V. REFERENCES

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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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