

Human Recombinant KiSS1-derived Peptide Receptor Stable Cell Line**Cat. No. M00190****Version 05282014**

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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

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, 10% DMSO

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

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

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.

§: 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.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Metastin in CHO-K1/KiSS1 and CHO-K1 cells

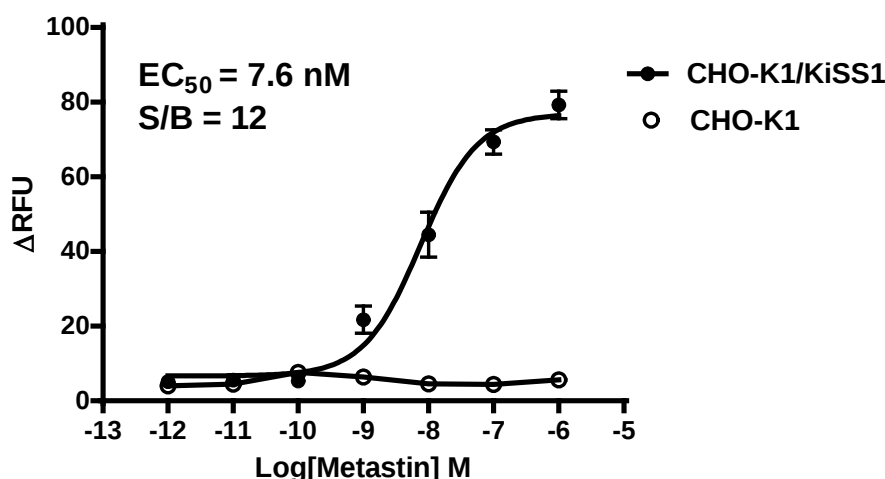


Figure 1. Metastin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/KiSS1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a KiSS1 receptor agonist, Metastin. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Metastin (Mean $\pm$ SD, n = 2). The EC₅₀ of Metastin on KiSS1 in CHO-K1 cells was 7.6 nM. The S/B of Metastin on KiSS in CHO-K1 cells was 12.

Notes:

- 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
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.
- Grow the cells in incubator with 37°C, 5 %CO₂.
- 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 %CO₂.

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

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.
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