

Human Recombinant MC5 Melanocortin Receptor Stable Cell Line

Cat. No. M00243

Version 05229014

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I. INTRODUCTION

Catalog Number: M00243

Cell Line Name: CHO-K1/MC5/Gα15

Gene Synonyms: MC5R

Expressed Gene: Genbank Accession Number NM_005913; no expressed tags

Host Cell: CHO-K/Gα15

Quantity: 2 vial (3×10⁶ per vial) frozen cells

Stability: 16 passages

Application: Functional assay for MC5 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

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

Culture Medium: Ham's F12, 10% FBS, 400 µg/ml G418, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The melanocortin receptor 5, MC5 receptor, is Gs-coupled GPCRs expressed in the adrenal gland, fat cells, kidney, leukocytes, lung, lymph node, mammary gland, ovary, pituitary, testis and uterus. Mice with targeted deletion of the MC5 receptor have a defect in water and thermoregulation secondary to their decreased production of sebaceous lipids.

§: 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 NDP-a-MSH in CHO-K1/MC5/Gα15 and CHO-K1/Gα15 cells

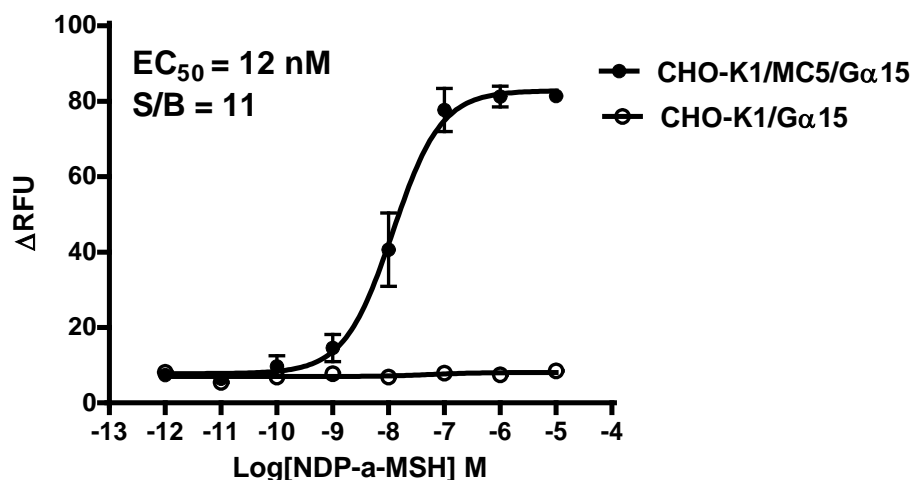


Figure 1. NDP-a-MSH-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MC5/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an MC3 receptor agonist, NDP-a-MSH. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NDP-a-MSH (Mean ± SD, n = 2). The EC₅₀ of NDP-a-MSH on MC5R co-expressing with Gα15 in CHO-K1 cells was 12 nM. The S/B of ACTH on MC3R co-expressing with Gα15 in CHO-K1 cells was 11.

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.

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 %CO₂.

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

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Chhajlani V. (1996) Distribution of cDNA for melanocortin receptor subtypes in human tissues. *Biochem Mol Biol Int.* 38(1):73-80.
2. Yang YK *et al*, (2001) Functional properties of an agouti signaling protein variant and characteristics of its cognate radioligand. *Am J Physiol Regul Integr Comp Physiol.* 281(6):R1877-86.
3. Chen W *et al*, (1997) Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides. *Cell.* 91(6):789-98.

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