Human Recombinant Melanin-concentrating Hormone Receptor 1 Stable Cell Line

Cat. No. M00211 **Version 05292014**

I. INTRODUCTION

Catalog Number: M00211

Cell Line Name: 293/MCH1

Expressed Gene: GenBank Accession Number NM_005297; no expressed tags

Host Cell: 293

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Applications: Functional assays for MCH1 receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 200 μg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The protein encoded by this gene is a member of the G protein-coupled receptor family 1. It is also an integral plasma membrane protein that binds melanin-concentrating hormone. The encoded protein can inhibit cAMP accumulation and stimulate intracellular calcium flux, and is probably involved in the neuronal regulation of food consumption. Melanin-concentrating hormone (MCH) is an important neuropeptide hormone involved in multiple physiological processes. Peptide derivatives of MCH have been developed as tools to aid researches that involve potent radioligands, receptor selective agonists, and potent antagonists. These tools have been used to further understand the role of MCH'sphysiology primarily in rodents.

§: 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 MCH in 293/MCH1 and 293 cells

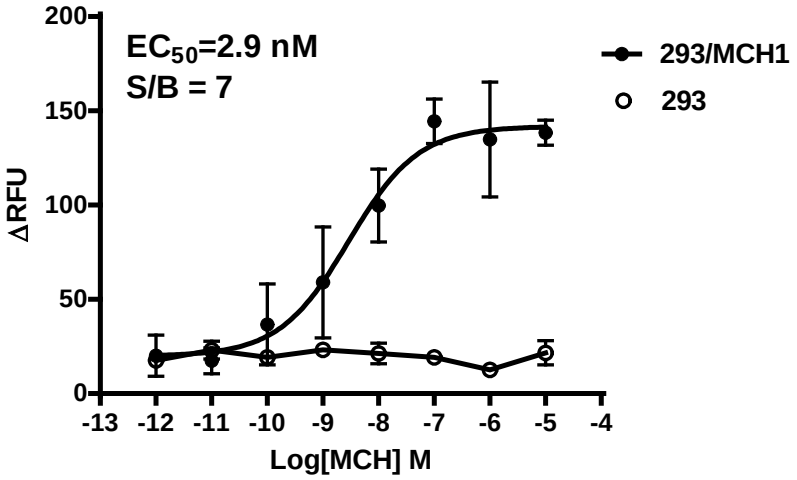

Figure 1. MCH-induced concentration-dependent stimulation of intracellular calcium mobilization in 293/MCH1 and 293 cells. The cells were loaded with Calcium-4 prior to stimulation with a MCH1 receptor agonist, MCH. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of MCH (Mean ± SD, n = 2). The EC_{50} of MCH on MCH1 in 293 cells was 2.9 nM. The S/B of MCH on MCH1in 293 cells was 7.

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.
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 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 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Gehlert DR (2009) Preclinical evaluation of melanin-concentrating hormone receptor 1 antagonism for the treatment of obesity and depression. *J Pharmacol Exp Ther.* 329(2):429-38.
2. Kolakowski LF (1997). Characterization of a human gene related to genes encoding somatostatin receptors. *FEBS Lett.* 398 (2-3): 253–8.

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