

Human Recombinant μ -Opioid Receptor OPRM1 Stable Cell Line

Cat. No. M00304

Version 06092014

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I. INTRODUCTION

Catalog Number: M00304

Cell Line Name: CHO-K1/OPRM1/G α 15

Gene Synonyms: MOR-1, MOR1, KIAA0403, OPRM

Expressed Gene: Genbank Accession Number NM_000914; no expressed tags

Host Cell: CHO-K1/G α 15Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for OPRM1 receptor

Freeze Medium: 45% Ham's F12, 45% FBS, 10% DMSO

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

Culture Medium: Ham's F12, 10% FBS, 200 μ g/ml Zeocin, 100 μ g/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The μ -opioid receptor (oprm1) is the principal site of action in the brain by which morphine, other opiate drugs of abuse, and endogenous opioid peptides effect analgesia and alter mood. Opioid receptors belong to the rhodopsin family of G protein-coupled receptors (GPCRs). The three types of opioid receptors (μ , δ , and κ) have been shown to associate with each other in a homotypic or heterotypic fashion when expressed in heterologous cells. A member of the seven-transmembrane domain (TM) G protein-coupled receptor (GPCR) superfamily, the μ -opioid receptor modulates ion channels and second messenger effectors in an opioid agonist-dependent fashion that is reversible by the classic opiate antagonist naloxone..

§: 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 DAMGO in CHO-K1/OPRM1/Gα15 and CHO-K1/Gα15 cells

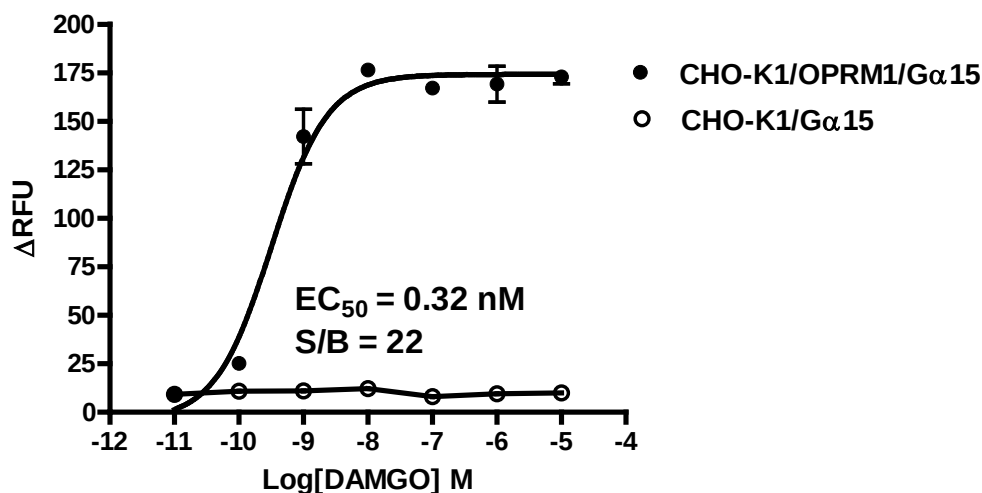


Figure 1. DAMGO-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OPRM1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a OPRM1 receptor agonist, DAMGO. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of DAMGO (Mean ± SD, n = 2). The EC₅₀ of DAMGO on OPRM1 co-expressing with Gα15 in CHO-K1 cells was 0.32 nM. The S/B of DAMGO on OPRM1 co-expressing with Gα15 in CHO-K1 cells was 22.

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.

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 %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. Kieffer, B. L., K. Befort, C. Gaveriaux-Ruff, and C. G. Hirth. (1992) The d-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. *Proc. Natl. Acad. Sci. USA* 89:12048–12052.
2. Mansour, A., Fox, C. A., Akil, H. and Watson, S. J. (1995) Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci.*, 18, 22 - 29.

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