Version: 02
Update (MM/DD/YYYY): 03/25/2025

Human Recombinant μ-Opioid Receptor Stable Cell Line
Cat. No. M00304

I. INTRODUCTION

Recombinant CHO-K1 cells stably overexpress human opioid receptor mu 1 (OPRM1) on the surface and contain high levels of G protein Gαi and Gαq to couple with the receptor in downstream signaling pathways.

Catalog Number: M00304
Cell Line Name: CHO-K1/OPRM1/Gα15
Gene Synonyms: LMOR, M-OR-1, MOP, MOR, MOR1, OPRM
Expressed Gene: NCBI reference sequence NM_000914; no expressed tags
Target Protein: NP_000905
Host Cell: CHO-K1
Size: Two vials of frozen cells (>1×10^6 per vial in 1 mL)
Culture Properties: Adherent
Freeze Medium: 45% Ham's F-12K (Kaighn's) (Cat. No. 21127, Life Technologies), 45% FBS (Cat. No. 10099-141, Life Technologies), 10% DMSO (Cat. No. D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 200 μg/ml Zeocin (Cat. No. R250-01, Life Technologies), 100 μg/ml Hygromycin B (Cat. No. 10687010, Life Technologies)
Stability: Stable through more than 16 passages with no significant changes in assay performance or expression profile.
Application: Calcium flux assay, IP-One assay and cAMP accumulation assay.
Mycoplasma Status: Negative. The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit (Cat. No. LT07-318, Lonza).
Storage: Store cells in liquid nitrogen immediately upon receipt. Thaw and recover cells within one year from the date received.

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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.

III. REPRESENTATIVE DATA

Calcium Flux Analysis

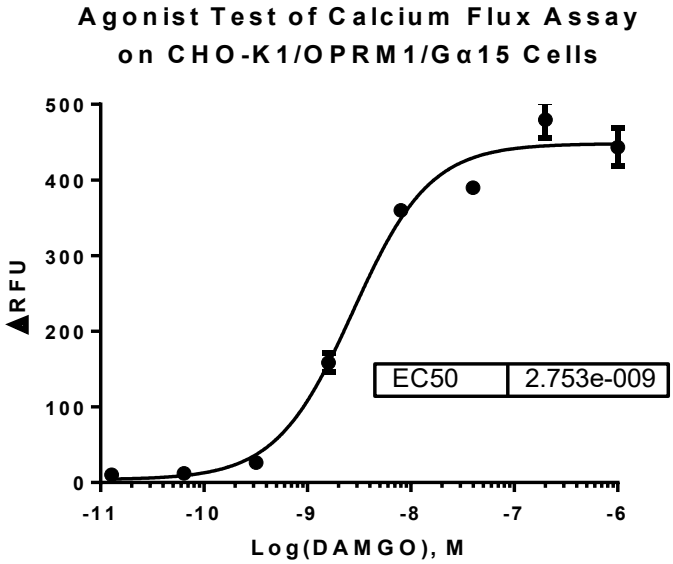

Figure 1. DAMGO-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OPRM1/Gα15 cells. The cells were loaded with Calcium-4 (Cat. No. R8142; Molecular Devices) prior to stimulation with OPRM1 agonist, DAMGO. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were recorded and normalized to plot against the log of the cumulative doses of DAMGO (mean ± SEM, n = 3). The EC_{50} of DAMGO on CHO-K1/OPRM1/Gα15 cells was 2.75 nM.

Notes:

EC_{50} value is calculated with four parameter logistic equation:

Y=Bottom + (Top-Bottom) / (1+10^ ((LogEC_{50}-X)*Hill Slope))

X is the logarithm of concentration. Y is the response.

Y is RFU and starts at Bottom and goes to Top along a sigmoid curve.

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cAMP Accumulation Assay

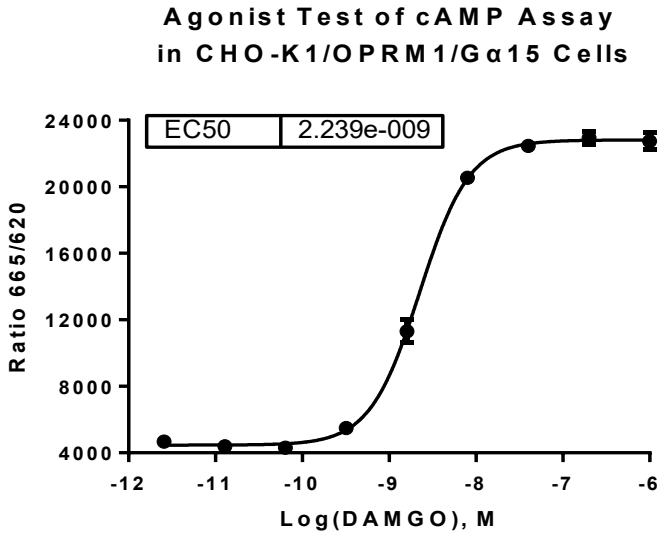

Figure 2. Dose dependent stimulation of intracellular cAMP accumulation upon treatment with DAMGO in CHO-K1/OPRM1/Gα15 cells. d2 acceptor fluorophore -labeled cAMP (Cat. No. 62AM4PEC; Revvity) and intracellular cAMP in CHO-K1/OPRM1/Gα15 cells competitively bind with Europium Cryptate-labeled anti-cAMP monoclonal antibody. The FRET signal decreases as the intracellular cAMP concentration rises and was measured by plate reader (Pherastar, BMG). The EC_{50} of DAMGO on CHO-K1/OPRM1/Gα15 cells was 2.24 nM.

IP-One Accumulation Assay

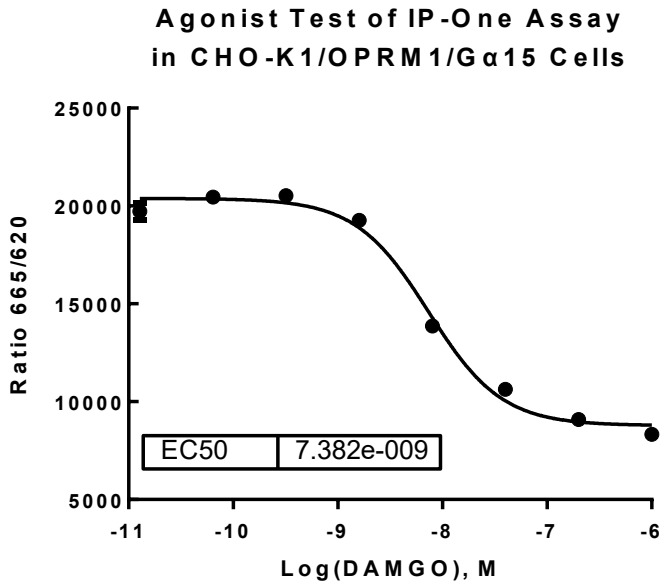

Figure 3. Dose dependent stimulation of intracellular IP-One accumulation upon treatment

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with DAMGO in CHO-K1/OPRM1/Gα15 cells. d2 acceptor fluorophore-labeled IP-One (Cat. No. 62IPAPEB; Revvity) and intracellular IP-One in CHO-K1/OPRM1/Gα15 cells competitively bind with Europium Cryptate-labeled anti-IP-One antibody. The FRET signal decreases as the intracellular IP-One concentration rises and was measured by plate reader (Pherastar, BMG). The EC_{50} of DAMGO on CHO-K1/OPRM1/Gα15 cells was 7.38 nM.

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial containing the frozen cells from liquid nitrogen tank and place into a 37°C water bath immediately.
2. Thaw the cells quickly (within 1-2 minutes) by gently swirling the vial. Do not vortex the cells.
3. When the cells are almost completely thawed, take the vial out of the water bath and decontaminate it with 70% ethanol.
4. In a biosafety hood, transfer the cells to a sterile 15 ml conical tube. Add 9 ml of complete growth medium to the cells.
5. Pellet cells by centrifugation at 200 × g for 3-5 minutes at room temperature.
6. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure that the cell pellet is not disturbed.
7. Resuspend the cells by gently flicking the tube. Gently add in 1 ml of complete growth medium.
8. Transfer the cell suspension into a 10 cm culture dish containing 10 ml of complete growth medium.
9. Grow the cells in an incubator at 37°C with 5% CO_2.
10. The cells will attach the dish in about 2-4 days. Check the status of the cells every day and don't disturb the cells till most cells attach well.
11. Change the medium with culture medium when cells grow well.

Sub-culturing Protocol

1. Remove the culture medium from the cells.
2. Wash cells with sterile PBS to remove all traces of serum which contains trypsin inhibitors.
3. Add 0.25% Trypsin/EDTA (Cat. No. 25200, Gibco) solution to the culture dish and observe the cells under an inverted microscope until the cell layer has dispersed (usually within 3-5 minutes).

   Notes: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. If cells are difficult to detach, place the dish in a 37°C incubator for about 2 minutes.
4. Add 6-8 ml of complete growth medium to the culture dish, aspirate the medium with cells by gentle pipetting and then add into a sterile falcon tube.
5. Centrifuge the cells at 200 x g for 5 minutes, and remove the medium.
6. Resuspend the cells in culture medium and add the cell suspension to a new culture dish.

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7. Grow the cells in an incubator at 37°C with 5% CO_2.

   Notes:

   Subcultivation Ratio: 1:3 to 1:8.

   Medium Renewal: Every 2 to 3 days.

Cryopreservation Protocol

1. Remove the cell culture medium, wash the cells with PBS once (optional), gently add enough trypsin to cover the cells and incubate for approximately 2 minutes in a 37°C incubator.
2. Resuspend in cell culture medium and transfer into a sterile 50 ml conical tube.
3. Count the viable cells using a hemocytometer. If preferred, also determine the cell viability. Cell viability should be at least 90% for good cryopreservation.
4. Centrifuge the cells at about 200 × g for 5 minutes at room temperature to pellet cells. Remove the supernatant gently without disturbing the cell pellet.
5. Resuspend cells by adding freezing medium to the tube to the required cell density (2-5 × 10^6 cells/ml for best results).
6. Aliquot 1 ml each into cryogenic storage vials and secure the lids.
7. Transfer the vials into a cryo-freezing container at room temperature and put into a -80°C freezer. The temperature inside the cryo-freezing container should decrease steadily by 1°C/minute.
8. After approximately 24 hours, remove the vials from the cyro-freezing container and transfer into liquid nitrogen for long term storage.

V. REFERENCES

1. Ho MK, New DC, Wong YH. (2002) Co-expressions of different opioid receptor types differentially modulate their signaling via G (16). *Neurosignals*, 11 (2): 115-22.
2. Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI, Reisine T. (1994) Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Mol Pharmacol*, 45 (2): 330-4.
3. Yu VC, Eiger S, Duan DS, Lameh J, Sadée W. (1990) Regulation of cyclic AMP by the mu-opioid receptor in human neuroblastoma SH-SY5Y cells. *J Neurochem*, 55 (4): 1390-6.

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