

Human Recombinant κ -Opioid Receptor OPRK1 Stable Cell Line

Cat. No. M00290

Version 06092014

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I. INTRODUCTION

Catalog Number: M00290

Cell Line Name: CHO-K1/OPRK1/G α 15

Gene Synonyms: OPRK1, OPRK, KOR-1, KOR

Expressed Gene: Genbank Accession Number NM_000912; 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 OPRK1 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, 100 μ g/ml Hygromycin B, 200 μ g/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. BACKGROUND

Opioid receptors and their endogenous peptide ligands play important roles in the reward and reinforcement of drugs such as heroin, cocaine, and alcohol. The κ -opioid receptor is a type of opioid receptor which binds the peptide opioid dynorphin as the primary endogenous ligand. κ -opioid receptors are widely distributed in the brain, spinal cord, and in pain neurons. They are associated with the risk for alcohol dependence.

§: 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 U-50488 in CHO-K1/OPRK1/G α 15 and CHO-K1/G α 15 cells

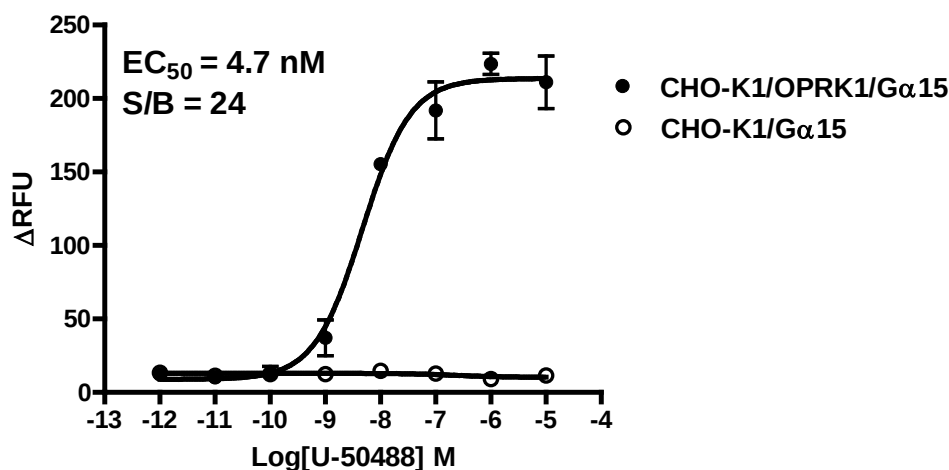


Figure 1. U-50488-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OPRK1/G α 15 and CHO-K1/G α 15 cells. The cells were loaded with Calcium-4 prior to stimulation with a OPRK1 receptor agonist, U-50488. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of U-50488 (Mean $\pm$ SD, n = 2). The EC₅₀ of U-50488 on OPRK1 co-expressing with G α 15 in CHO-K1 cells was 4.7 nM. The S/B of U-50488 on OPRK1 co-expressing with G α 15 in CHO-K1 cells was 24.

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.

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. Zhu, J., Luo, L. Y., Li, J. G., Chen, C. and Liu-Chen, L. Y. (1997) Activation of the cloned human kappa opioid receptor by agonists enhances [³⁵S]GTPgammaS binding to membranes: determination of potencies and efficacies of ligands, *J. Pharmacol. Exp. Ther.*, 282, 676 - 684.
2. Chavkin, C., Sud, S., Jin, W., Stewart, J., Zjawiony, J. K., Siebert, D. J., Toth, B. A., Hufeisen, S. J. and Roth, B. L. (2004) Salvinorin A, an active component of the hallucinogenic sage salvia divinorum is a highly efficacious kappa-opioid receptor agonist: structural and functional considerations. *J. Pharmacol. Exp. Ther.*, 308, 1197 - 1203.

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