Human Recombinant δ-Opioid Receptor OPRD1 Stable Cell Line

Cat. No. M00323 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00323
Cell Line Name: CHO-K1/OPRD1/Gα15
Gene Synonyms: OPRD1; OPRD
Expressed Gene: Genbank Accession Number NM_000911; no expressed tags
Host Cell: CHO-K1/Gα15
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for OPRD1 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, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Opioid receptor family includes three classic receptors, μ, δ, and κ, also known as OP1, OP2 and OP3, respectively. The receptors are $G_{i/o}$-coupled GPCRs which will reduce intracellular cAMP levels after activation. δ-opioid receptor modulates many kinase cascades including ERKs, Akts, JNKs, STAT3, P38 involving Src, Ras, Rac, Raf-1, Cdc42, RTKs. In addition, δ-opioid receptor has also been proposed to interact with μ receptors. The observed pharmacological cross-talk may partially arise from agonist cross-reactivity.

§: 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 SNC80 in CHO-K1/OPRD1/Gα15 and CHO-K1/Gα15 cells

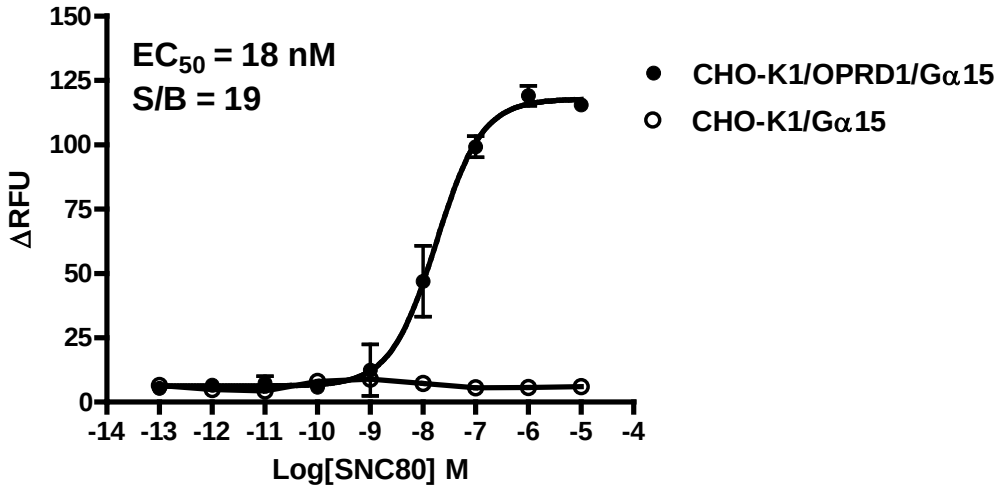

Figure 1. SNC80-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OPRD1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an OPRD1 receptor agonist, SNC80. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of SNC80 (Mean ± SD, n = 2). The EC_{50} of SNC80 on OPRD1 co-expressing with Gα15 in CHO-K1 cells was 18 nM. The S/B of SNC80 on OPRD1 co-expressing with Gα15 in CHO-K1 cells was 19.

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

Radioligand Binding Assay

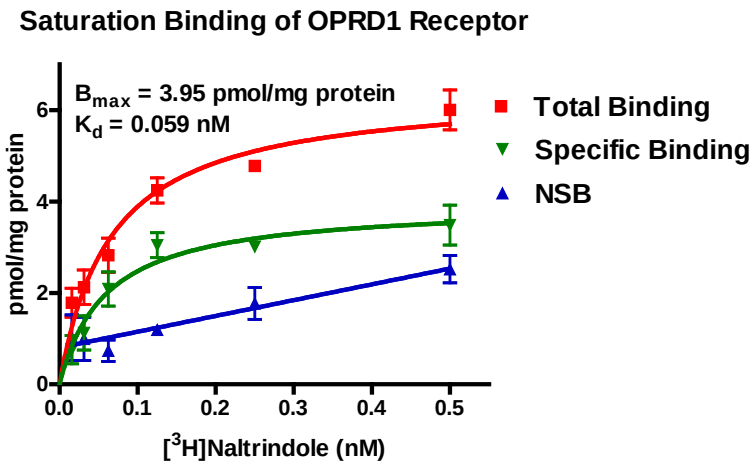

Figure 2. 5 µg of membranes prepared from CHO-K1 cells stably expressing OPRD1 receptors were incubated with indicated concentrations of [^{3}H]Naltrindole in the absence (total binding) or presence of 1000-fold excess unlabeled Naloxone (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

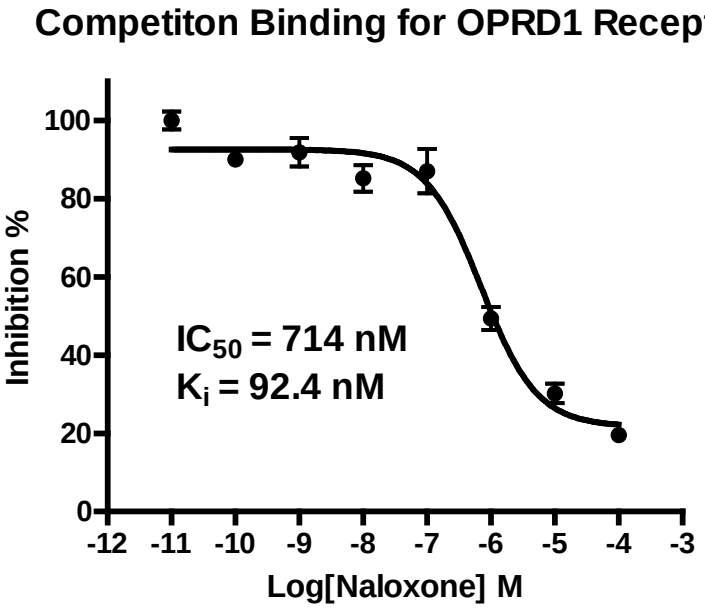

Figure 3. 5 µg of membranes prepared from CHO-K1 cells stably expressing OPRD1 receptors were incubated with indicated concentrations of Naloxone in the presence of 0.4 nM [^{3}H]Naltrindole. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

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. 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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