Human Recombinant δ-Opioid Receptor OPRD1 Stable Cell Line
Cat. No. M00323 **Version 07292020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for OPRD1 receptor (Calcium flux assay, cAMP assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 200 μg/ml Zeocin (Cat. #R250-01, Life Technologies), 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

Calcium mobilization assay

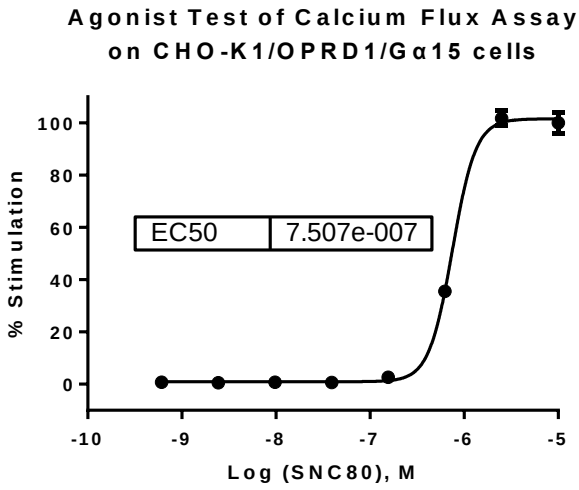

Figure 1. SNC80-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OPRD1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist SNC80. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of SNC80 (Mean ± SD, n = 2). The EC_{50} of SNC80 on this cell was 0.75 µM.

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

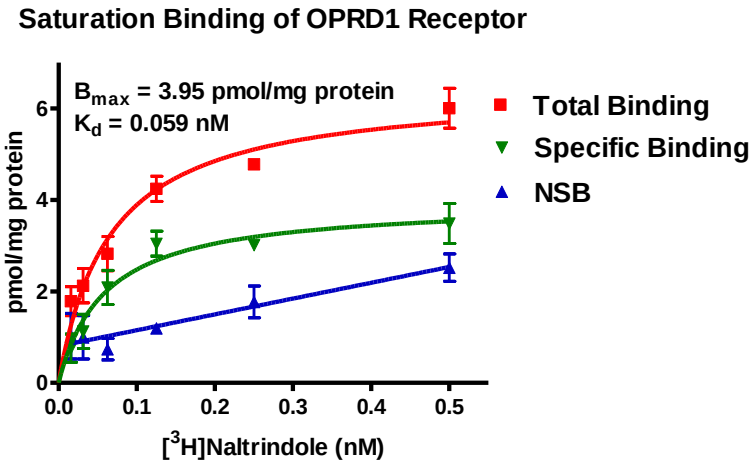

Figure 2. 5 µg of membranes prepared from CHO-K1 cells stably expressing OPRD1 receptors

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.

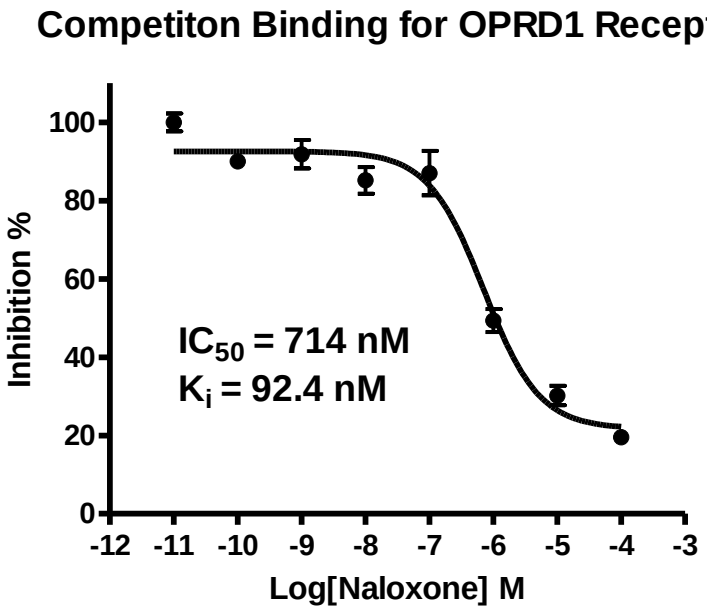

Figure 3. 5 µg of membranes prepared from CHO-K1 cells stably expressing OPRD1 receptors were incubated with indicated concentrations of Naloxone in the presence of 0.4 nM [^{3}H]Naltrindole. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear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