Human Recombinant Opiate Receptor-like 1 Stable Cell Line
Cat. No. M00447 **Version 07312020**

I. Introduction ……1
II. Background ……1
III. Representative Data …… 2
IV. Thawing and Subculturing …… 2
V. References …… 3
VI. Limited Use License Agreement…… 4

I. INTRODUCTION

Catalog Number: M00447
Cell Line Name: CHO-K1/OPRL1/Gα15
Gene Synonyms: OPRL1; KOR-3; MGC34578; NOCIR; OOR; ORL1
Expressed Gene: Genbank Accession Number NM_000913; 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 OPRL1 receptor (Calcium flux 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, 400 µg/ml G418 (Cat. #10131-035, Gibco), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

Following the cloning of the classical opioid receptors (mu, delta and kappa), the opiate receptor like-1 (ORL1) was identified as a G-protein coupled receptor (GPCR) with 65% structure homology to the other members of the opioid family. OPRL1 is a receptor for the 17 aa neuropeptide nociceptin/orphanin FQ and may be involved in the regulation of numerous brain activities, particularly instinctive and emotional behaviors. Recently, new study results are consistent with the reported high density of ORL1 receptor mRNA in dorsal raphe nucleus and with inhibitory actions of nociceptin in cells expressing ORL1.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Orphanin FQ in CHO-K1/OPRL1/Gα15 cells

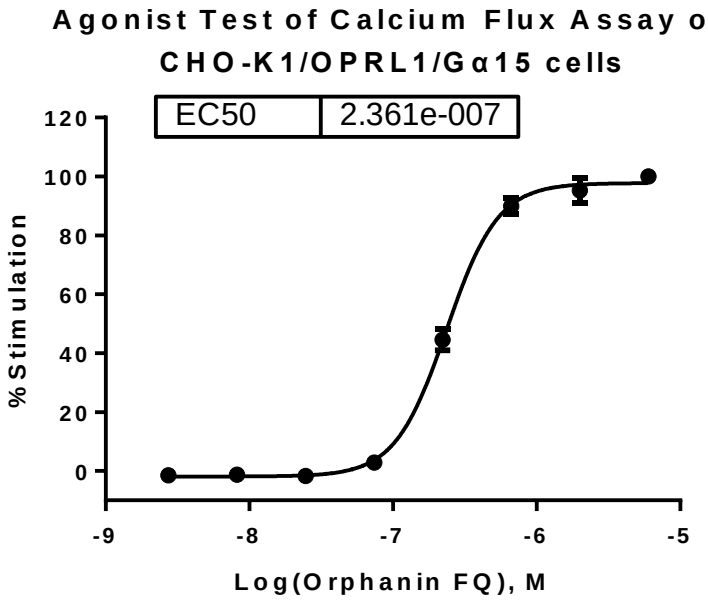

Figure 1. Orphanin FQ-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/OPRL1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with Orphanin FQ. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of Orphanin FQ (Mean ± SEM, n = 3). The EC_{50} of Orphanin FQ on this cell was 236.1 nM.

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

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. Lachowicz JE, Shen Y, Monsma FJ, et al. (1995) Molecular cloning of a novel G protein-coupled receptor related to the opiate receptor family. *J Neurochem.* 64(1):34–40.
2. W. Vaughan, M. J. Christie. (1996) Increase by the ORL1 receptor (opioid receptor-like1) ligand, nociceptin, of inwardly rectifying K conductance in dorsal raphe nucleus neurones. *Br J Pharmacol.* 117(8): 1609–1611

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