Human Recombinant Neuropeptide FF Receptor 1 Stable Cell Line

Technical Manual No. TM0559 **Version 10132010**

I. Introduction

Catalog Number: M00431

Cell Line Name: CHO-K1/NPFF1/Gα15

Gene Synonyms: NPFFR1; FLJ10751; GPR147; NPFF1; NPFF1R1; OT7T022

Expressed Gene: Genbank Accession Number NM_022146; 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 NPFF1 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

The neuropeptide FF receptors are members of the G-protein coupled receptor superfamily of integral membrane proteins which bind the pain modulatory neuropeptides AF and FF. These neuropeptides are thought to be involved in modulation of opioid receptor function in the brain and spinal cord, and can either reduce or increase opioid receptor function depend which tissue they are released in, reflecting a complex role for neuropeptide FF in pain responses.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by RFRP-3 (human) in CHO-K1/NPFF1/Gα15 and CHO-K1/Gα15 cells

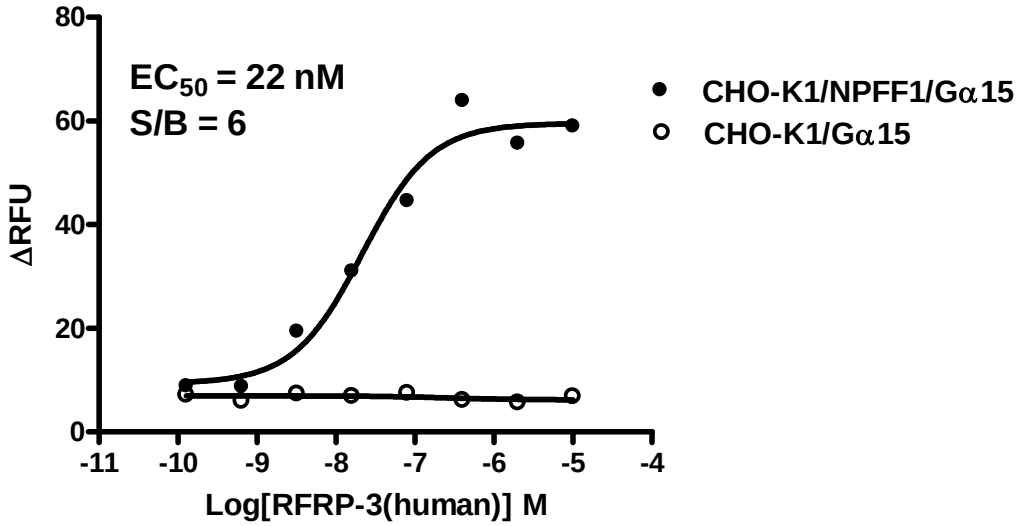

Figure 1. RFRP-3-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NPFF1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an NPFF1 receptor agonist, RFRP-3. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of RFRP-3 (Mean ± SD, n = 2). The EC_{50} of RFRP-3 on NPFF1 co-expressing with Gα15 in CHO-K1 cells was 22 nM. The S/B of RFRP-3 on NPFF1 co-expressing with Gα15 in CHO-K1 cells was 6.

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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B and Zeocin to concentrations of 100 μg/ml and 200 μg/ml respectively the following day.

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Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References

1. Elshourbagy NA, Ames RS, Fitzgerald LR, et al., Receptor for the pain modulatory neuropeptides FF and AF is an orphan G protein-coupled receptor (2000). *J. Biol. Chem*. 275 (34): 25965–71.
2. Yang HY, Iadarola MJ, Modulatory roles of the NPFF system in pain mechanisms at the spinal level(2006). *Peptides.* 27 (5): 943–52.
3. Roumy M, Lorenzo C, Mazères S, et al., Physical association between neuropeptide FF and micro-opioid receptors as a possible molecular basis for anti-opioid activity(2007). *The Journal of Biological Chemistry.* 282 (11): 8332–42.
4. Yang HY, Tao T, Iadarola MJ, Modulatory role of neuropeptide FF system in nociception and opiate analgesia(2008). *Neuropeptides.* 42 (1): 1–18.

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