Human Recombinant Niacin Receptor 1 Stable Cell Line
Cat. No. M00428 **Version 07302020**

I. INTRODUCTION

Catalog Number: M00428
Cell Line Name: CHO-K1/NIACR1/Gα15
Gene Synonyms: NIACR1; GPR109A; PUMAG; Puma-g; HM74a; HM74b
Expressed Gene: Genbank Accession Number NM_000685; 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 NIACR1 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, 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

GPR109A is a high affinity receptor for nicotinic acid (niacin) and is a member of the nicotinic acid receptor family of G protein-coupled receptors (the other identified member being GPR109B). GPR109A is a $G_{i/o}$ protein-coupled receptor with high affinity for nicotinic acid. In GPR109A knockout mice, the effects of niacin on both lipids and flushing are eliminated. Furthermore in arrestin beta 1 knockout mice, niacin's effect on flushing is greatly reduced while the lipid modifying effects are maintained. GPR109A is believed to be an important biomolecular target of niacin which is a widely prescribed drug for the treatment of dyslipidemia and to increase HDL cholesterol but whose therapeutic use is limited by flushing.

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

III. REPRESENTATIVE DATA

Calcium mobilization assay:

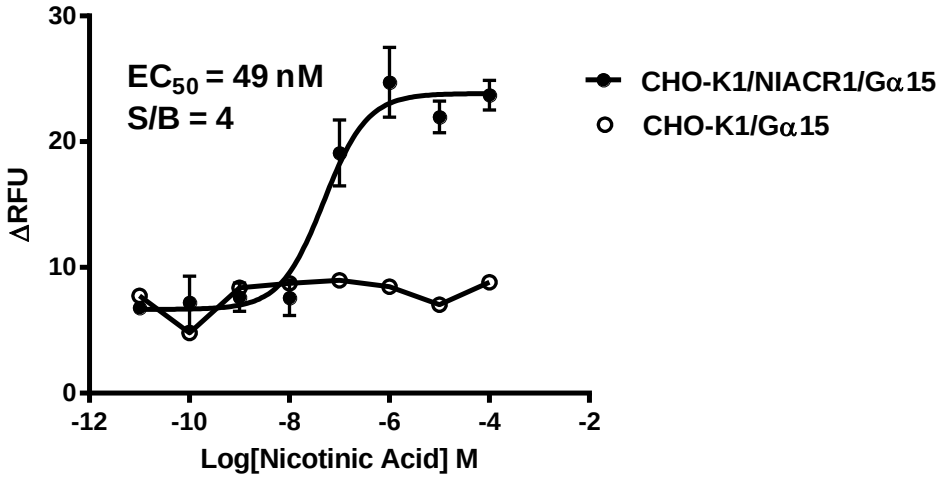

Figure 1. Nicotinic Acid-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NIACR1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an NIACR1 receptor agonist, Nicotinic Acid. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Nicotinic Acid (Mean ± SD, n = 2). The EC_{50} of Nicotinic Acid on NIACR1 co-expressing with Gα15 in CHO-K1 cells was 49 nM. The S/B of Nicotinic Acid on NIACR1 co-expressing with Gα15 in CHO-K1 cells was 4.

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. Wise A, Foord SM, Fraser NJ, *et al.* (2003) Molecular identification of high and low affinity receptors for nicotinic acid. *The Journal of Biological Chemistry*. 278(11): 9869–74.
2. Soga T, Kamohara M, Takasaki J, *et al.* (2003) Molecular identification of nicotinic acid receptor. *Biochemical and Biophysical Research Communications*. 303(1): 364–9.
3. Pike NB, (2005) Flushing out the role of GPR109A (HM74A) in the clinical efficacy of nicotinic acid. *The Journal of Clinical Investigation*. 115(12): 3400–3.
4. Tunaru S, Kero J, Schaub A (2003) PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. *Nature Medicine*. 9(3): 352–5.
5. Benyó Z, Gille A, Kero J, *et al.* (2005) GPR109A (PUMA-G/HM74A) mediates nicotinic acid-induced flushing. *The Journal of Clinical Investigation*. 115(12): 3634–40.

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