Human Recombinant Apelin Receptor Stable Cell Line
Cat. No. M00245 **Version 07272020**

I. INTRODUCTION

Catalog Number: M00245
Cell Line Name: CHO-K1/AGTRL1/Gα15
Gene Synonyms: APJ; APLNR; FLJ90771; MGC45246; AGTRL1; HG11; APJR; FLJ96609
Expressed Gene: Genbank Accession Number NM_005161; no expressed tags
Host Cell: CHO-K1/Gα15
Quantity: 2 vial (>1×10^6 per vial) frozen cells
Stability: More than 16 passages
Application: Functional assay for AGTRL1 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-12 K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12 K (Kaighn's), 10% FBS, 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen), 400 μg/ml G418 (Cat. #10131-035, Gibco)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

The apelin receptor AGTRL1 is G_i/G_o-coupled GPCRs expressed in the heart, coronary artery, aorta, internal mammary artery, pulmonary artery and saphenous vein, it also expressed in lung, kidney and adrenal gland. In AGTRL1 receptor knockout mice baseline blood pressure is not changed compared to wild-type animals. Vasoconstrictor responses to angiotensin II however are significantly more pronounced in the knockout animals.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Apelin-13 in CHO-K1/AGTRL1/Gα15 cells

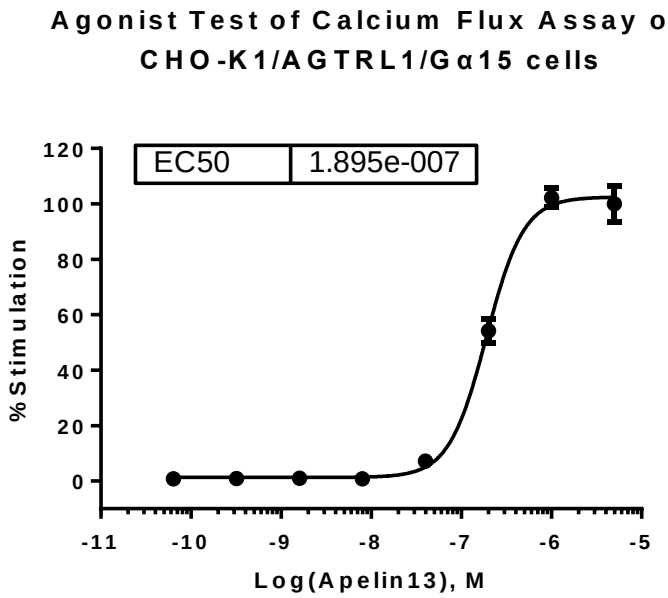

Figure 1. Apelin-13-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/AGTRL1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with an AGTRL1 receptor agonist, Apelin-13.The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (10-fold dilution) of Apelin-13 (Mean ± SD, n = 2). The EC_{50} of Apelin-13 on this cells was 0.19 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.

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. Hosoya M, *et al.* (2000) Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. J Biol Chem. 275(28):21061-7.
2. Masri B, *et al.* (2002) Apelin (65-77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. *Biochem Biophys Res Commun.* 290(1):539-45
3. Kawamata Y, *et al.* (2001) Molecular properties of apelin: tissue distribution and receptor binding. *Biochim Biophys Acta.* 1538(2-3):162-71
4. Ishida J, *et al.* (2004) Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. *J Biol Chem.* 279(25):26274-9. Epub 2004 Apr 15

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