Human Recombinant GAL1 Galanin Receptor Stable Cell Line
Cat. No. M00277 **Version 07282020**

I. INTRODUCTION

Catalog Number: M00277
Cell Line Name: CHO-K1/GALR1/Gα15
Expressed Gene: GenBank Accession Number NM_001480; no expressed tags
Host Cell: CHO-K1
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assays for GAL1 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

The neuropeptide galanin elicits a range of biological effects by interacting with specific G-protein-coupled receptors. Galanin receptors are seven-transmembrane proteins that have been shown to activate a variety of intracellular second-messenger pathways. GALR1 inhibits adenylyl cyclase via a G protein of the Gi/Go family. GALR1 is widely expressed in the brain, spinal cord, and peripheral sites including small intestine and heart.

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

III. REPRESENTATIVE DATA

Calcium Flux Assay

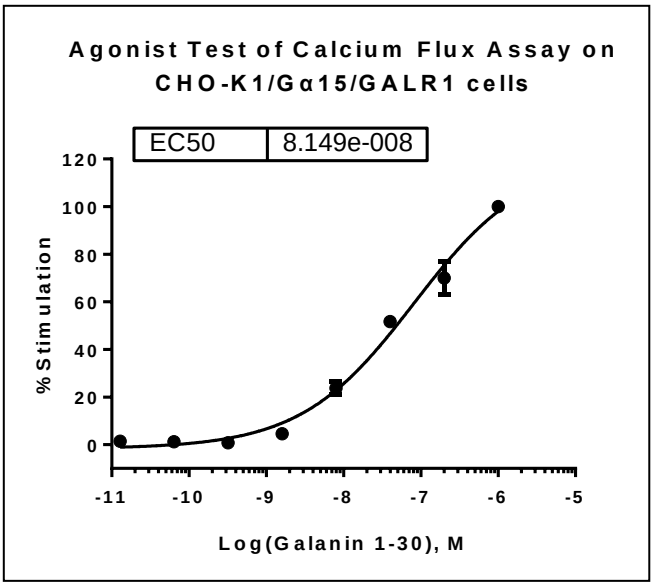

Figure 1: Galanin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GALR1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with galanin. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of galanin (Mean ± SD, n = 2). The EC_{50} of galanin on this cell was 81.5 nM.

Note:

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 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 for 5 min, and remove the medium.
4. Resuspend the cells with 1 ml complete growth medium.
5. Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
6. Transfer the dish into an incubator of 37°C, 5% CO_2.
7. Add antibiotic into the medium on the next 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 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 during incubation. 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 complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 X g for 5min, and remove the medium.
6. Resuspend the cells in culture medium and transfer the cells to a new culture dish.
7. Transfer the dish into an incubator of 37°C, 5% CO_2.

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

V. REFERENCES

1. Burgevin MC, Loquet I, Quarteronet D, Habert-Ortoli E. (1995). Cloning, pharmacological characterization, and anatomical distribution of a rat cDNA encoding for a galanin receptor. *J Mol Neurosci*.6 (1):33-41.
2. Mitsukawa K, Lu X, Bartfai T. Galanin, galanin receptors and drug targets. (2008). *Cell Mol Life Sci*. Jun; 65 (12):1796-805.

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