Human Recombinant GAL2 Galanin Receptor Stable Cell Line

Cat. No. M00209 **Version 06232020**

I. INTRODUCTION

Catalog Number: M00209
Cell Line Name: CHO-K1/GALR2
Expressed Gene: GenBank Accession Number NM_003857; 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 GAL2 receptor
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, 400 μg/ml G418 (Cat. #10131-035, Gibco)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

Actions of neuropeptide galanin are mediated by three G-protein coupled receptors, GalR1, GalR2, and GalR3. Human GALR2 was shown to couple to phospholipase C and to be involved in the elevation of intracellular calcium levels. GALR2 mRNA shows a wide distribution in the brain (mammillary nuclei, dentate gyrus, cingulate gyrus, posterior hypothalamic, supraoptic, and arcuate nuclei) and restricted peripheral tissue distribution with highest mRNA levels detected in human small intestine. Galanin has been implicated in anxiety-related behaviors, cognition, analgesia, and feeding in rodents.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by galanin in CHO-K1/GALR2 cells

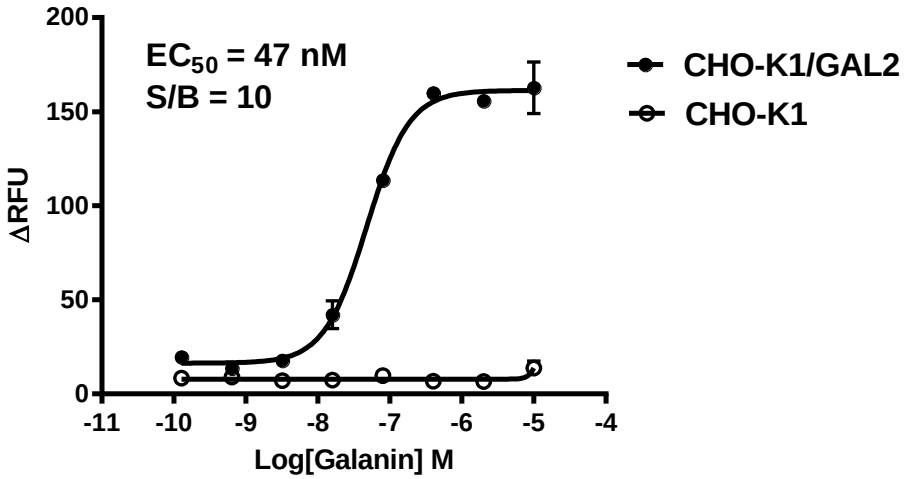

Figure 1: Galanin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GALR2 cells. The cells were loaded with Calcium-4 prior to being stimulated with a GAL2 receptor agonist, galanin. The intracellular calcium change was measured by FLIPR^{TETRA}.The relative 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 cells was 47 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. Kolakowski LF Jr, (1998) Molecular characterization and expression of cloned human galanin receptors GALR2 and GALR3.*J Neurochem*.71 (6):2239-51
2. Bailey KR, (2007) Galanin receptor subtype 2 (GalR2) null mutant mice display an anxiogenic-like phenotype specific to the elevated plus-maze. *Pharmacol Biochem Behav*, 86(1):8-20

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