

Human Recombinant GAL2 Galanin Receptor Stable Cell Line**Cat. No. M00209****Version 05272014**

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I. INTRODUCTION

Catalog Number: M00209

Cell Line Name: CHO-K1/GAL2

Expressed Gene: GenBank Accession Number NM_003857; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Applications: Functional assays for GAL2 receptor

Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 400 μ g/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

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.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritidis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Galanin in CHO-K1/GAL2 and CHO-K1 cells

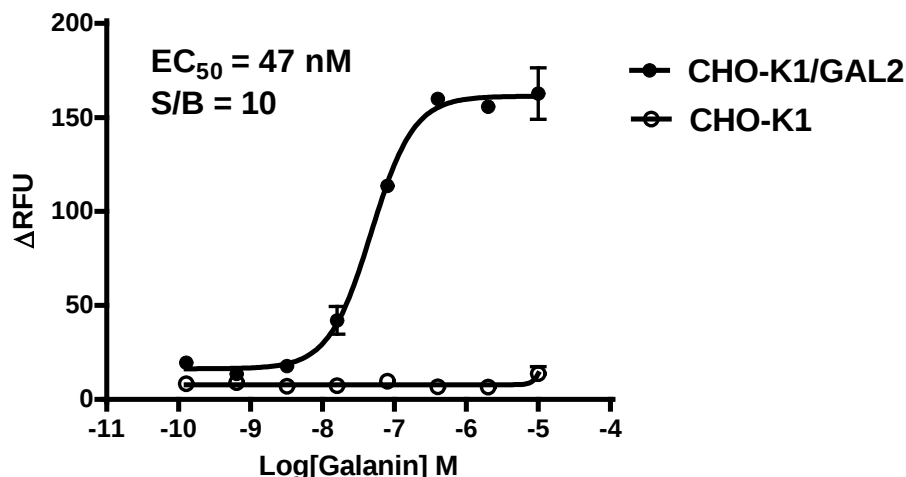


Figure 1. Galanin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GAL2 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a GAL2 receptor agonist, Galanin. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Galanin (Mean $\pm$ SD, $n = 2$). The EC_{50} of Galanin on GAL2 in CHO-K1 cells was 47 nM. The S/B of Galanin on GAL2 in CHO-K1 cells was 10.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{Log}EC_{50} - X) \cdot \text{HillSlope}})$$

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 %CO₂.
7. Add antibiotic in the following day.

Sub-culturing Protocol

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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 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

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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