

Human Recombinant GAL1 Galanin Receptor Stable Cell Line

Cat. No. M00277

Version 05262014

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I. INTRODUCTION

Catalog Number: M00277

Cell Line Name: CHO-K1/GAL1/Gα15

Expressed Gene: GenBank Accession Number NM_001480; 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 GAL1 receptor

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

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

Culture Medium: Ham's F12, 10% FBS, 200 µg/ml Zeocin, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

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.

§: 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/GAL1/Gα15 and CHO-K1/Gα15 cells

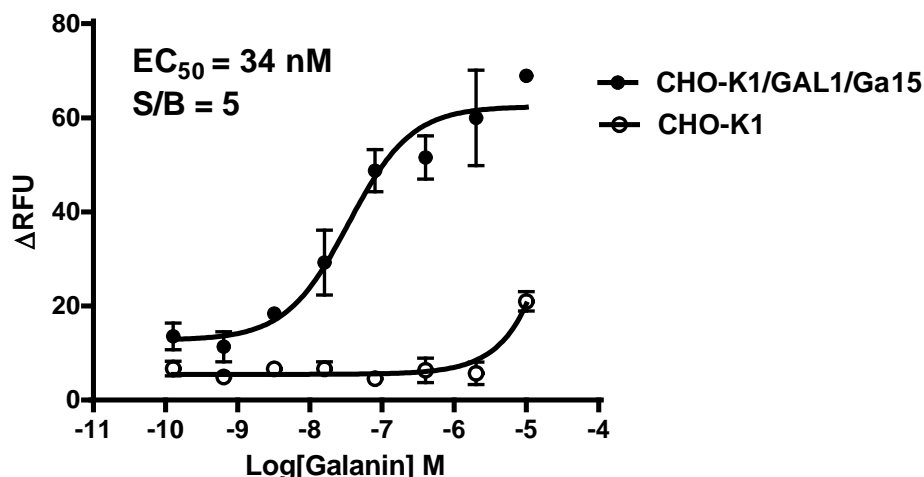


Figure 1. Galanin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GAL1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a GAL1 receptor agonist, Galanin. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Galanin (Mean ± SD, n = 2). The EC₅₀ of Galanin on GAL1 co-expressing with Gα15 in CHO-K1 cells was 34 nM. The S/B of Galanin on GAL1 co-expressing with Gα15 in CHO-K1 cells was 5.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

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

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.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- 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.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
- Grow the cells in incubator with 37°C, 5 %CO₂.
- 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 %CO₂.

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

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.

GenScript USA Inc,

860 Centennial Ave.

Piscataway, NJ 08854

Toll-Free: 1-877-436-7274

Tel: 1-732-885-9188, Fax: 1-732-210-0262

Email: product@genscript.com

Web: <http://www.genscript.com>

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860 Centennial Ave., Piscataway, NJ 08854, USA

Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

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