Human Recombinant Metabotropic Glutamate Receptor 2 Stable Cell Line

Cat. No. M00427 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00427
Cell Line Name: CHO-K1/GRM2/Gα15
Gene Synonyms: GRM2; GPRC1B; mGLUR2; mGlu2
Expressed Gene: Genbank Accession Number NM_000839; no expressed tags
Host Cell: CHO-K1/Gα15
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for GRM2 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

L-glutamate is the major excitatory neurotransmitter in the central nervous system and activates both ionotropic and metabotropic glutamate receptors. Glutamatergic neurotransmission is involved in most aspects of normal brain function and can be perturbed in many neuropathologic conditions. GRM2 is linked to the inhibition of the cyclic AMP cascade, included in group II metabotropic glutamate receptors.

§: 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 L-Glutamate in CHO-K1/GRM2/Gα15 and CHO-K1/Gα15 cells

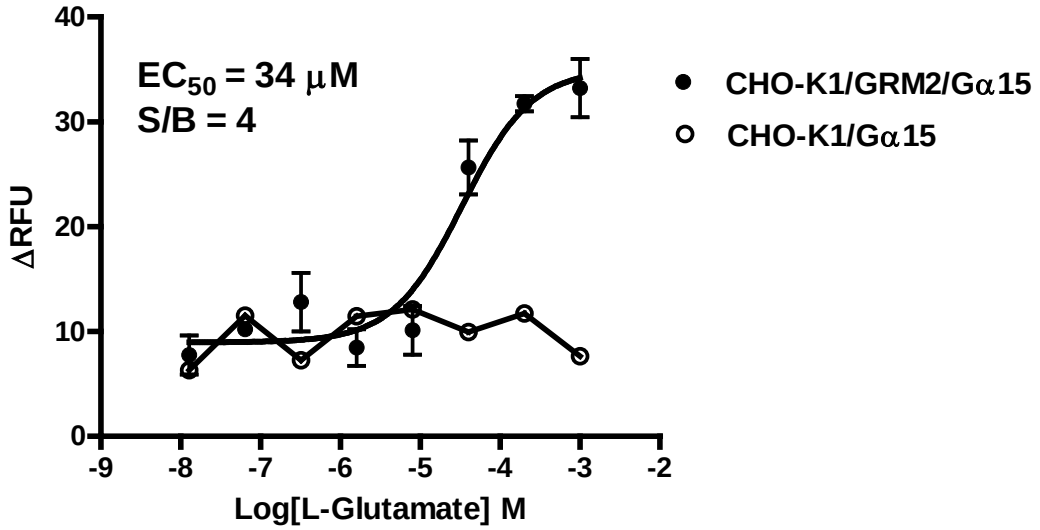

Figure 1. L-Glutamate-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GRM2/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a GRM2 receptor agonist, L-Glutamate. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of L-Glutamate (Mean ± SD, n = 2). The EC_{50} of L-Glutamate on GRM2 co-expressing with Gα15 in CHO-K1 cells was 34 μM. The S/B of L-Glutamate on GRM2 co-expressing with Gα15 in CHO-K1 cells was 4.

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.
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 %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 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Flor PJ, Lindauer K, Puttner I, *et al*. (1995) Molecular cloning, functional expression and pharmacological characterization of the human metabotropic glutamate receptor type 2. *Eur J Neurosci.* 7(4): 622–9.
2. D'Alessandro PL, Corti C, Roth A, *et al.* (2010) The identification of structurally novel, selective, orally bioavailable positive modulators of mGluR2. *Bioorg. Med. Chem. Lett.*. 20(2): 759–62.

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