Human Recombinant G-Protein Coupled Receptor 68 Stable Cell Line

Cat. No. M00439 **Version 08152016**

I. INTRODUCTION

Catalog Number: M00439

Cell Line Name: CHO-K1/GPR68/$G_{\alpha 15}$

Gene Synonyms: GPR68; MGC111379; OGR1

Expressed Gene: Genbank Accession Number NM_003485; no expressed tags

Host Cell: CHO-K1/$G_{\alpha 15}$

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

Stability: 16 passages

Application: Functional assay for GPR68 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, 100 μg/ml Hygromycin B, 200 μg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The GPR68 is a proton-sensing receptor involved in pH homeostasis. A study revealed that in osteosarcoma cells and primary human osteoblast precursors which expression of GPR68 exhibit strong PH-dependent inositol phosphate formation.

§: 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

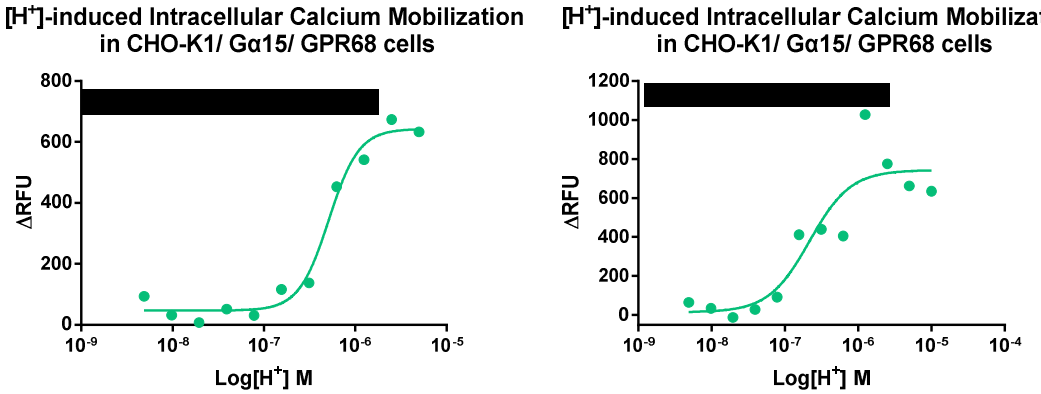

Figure 1. H^+-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GPR68/$G_{\alpha 15}$. The cells were loaded with Calcium-4 prior to stimulation with a GPR68 receptor agonist, H^+. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses of H^+ (Mean ± SD, n = 2). The EC_{50} of H^+ on GPR68 co-expressing with $G_{\alpha 15}$ in CHO-K1 cells was pH 6.29 (n=1 test) and pH6.66 (n=2 test).

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 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 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 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. Xu, Y., Casey, G. (1996) Identification of human OGR1, a novel G protein-coupled receptor that maps to chromosome 14. *Genomics.* 35, 397–402
2. Ludwig, M.G *et al.* (2003) Proton-sensing G-protein-coupled receptors. *Nature.* 425:93–98

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