Human Recombinant Bile Acid Receptor 1 Stable Cell Line

Cat. No. M00432 **Version 05232014**

I. INTRODUCTION

Catalog Number: M00432

Cell Line Name: CHO-K1/GPBAR1

Gene Synonyms: BG37, M-BAR, GPR131, hGPCR19, and TGR5

Expressed Gene: Genbank Accession Number NM_170699; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for GPBAR1 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

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The gene GPBRA1 encodes G protein-coupled bile acid receptor. Bile acid-binding induces its internalization, activation of extracellular signal-regulated kinase and intracellular cAMP production.

§: 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.

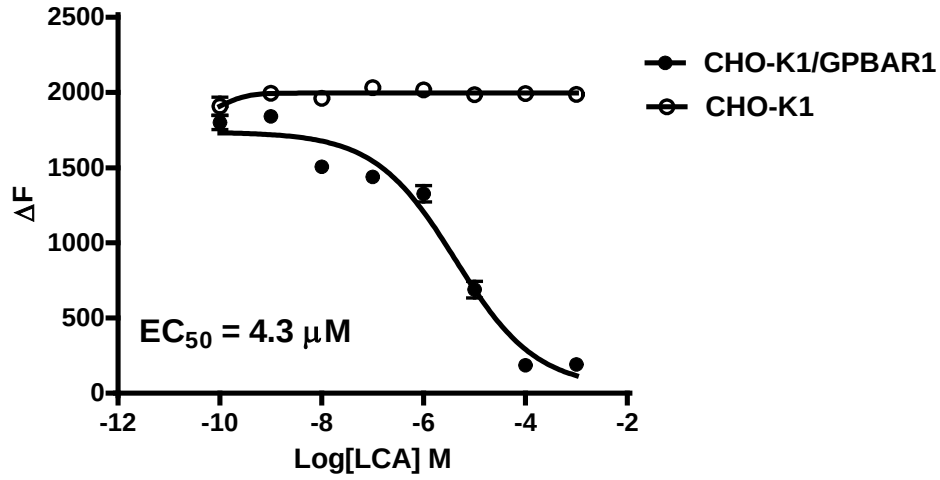
III. ASSAY DEVELOPMENT

This cell based assay is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). It is a competitive immunoassay that uses cAMP labeled with the d2 acceptor flourophore and an anti-cAMP monoclonal AB labeled with Europium Cryptate. The FRET signal decreases as cAMP concentration rises.

Agonist Assay Protocol

1. Seed 5 µl CHO-K1/GPBAR1 cells into a 384-well low volume plate, 4,000 cells per well.
2. Add 5 µl compound or lithocholic acid (LCA) (diluted in buffer with 2% DMSO) to each well and incubate the plate for 30 min at 23°C.
3. Add 5ul of cAMP-d2 lysis buffer solution to each well.
4. Add 5µl of cAMP-AB lysis buffer solution to each well.
5. Incubate the plate in the dark for one hour at 23°C.
6. Read the plate PHERAstar PLUS (BMG Labtech, Offenburg, Germany).

Agonist Data



Data Analysis:

1. Ratio = A_{665nm}/B_{620nm} x 104
2. Mean Ratio = $\sum_{Ratio}/2$
3. Delta F
4. = (Sample ratio-$Ratio_{neg}$)/$Ratio_{neg}$ x 100
5. Signal to background Ratio(S/B) = $M_{known\ agonist}$ / $M_{DMSO\ control}$
6. CV = 100 x SD/M (%)
7. Z factor = 1 - 3 x | $SD_{known\ agonist}$ +$SD_{DMSO\ control}$ | / | $Mean_{known\ agonist}$ -$Mean_{DMSO\ control}$ |

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. In the following day, replace the cells with fresh medium contains antibiotic.

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. Maruyama *et al*. (2002) Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun* 298: 714-9.
2. Katsuma *et al*. (2005) Bile acids promote glucagon-like peptide-1secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem Biophys Res Commun* 329: 386-90.

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