Human Recombinant VPAC1 Receptor Stable Cell Line

Cat. No. M00342 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00342
Cell Line Name: CHO-K1/VPAC1/Gα15
Gene Synonyms: VIPR1, VIP1 receptor
Expressed Gene: Genbank Accession Number NM_004624; 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 VPAC1 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 widespread neuropeptide vasoactive intestinal peptide (VIP) has two receptors VPAC1 and VPAC2. The vasoactive intestinal polypeptide receptor VPAC1 is G_s-coupled GPCRs expressed in the lung, prostate, peripheral blood leukocytes, liver, brain, small intestine colon, heart, spleen, placenta, kidney, thymus, and testis.

§: 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 VIP in CHO-K1/VPAC1/Gα15 and CHO-K1/Gα15 cells

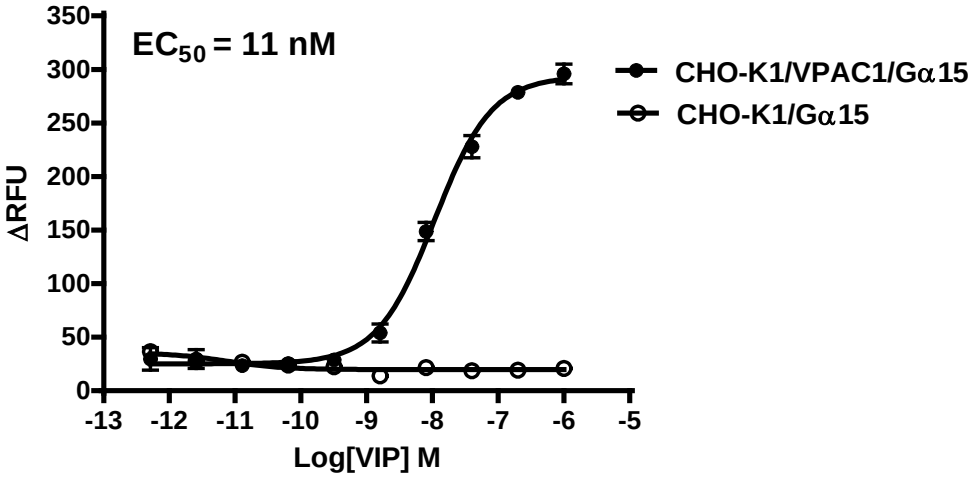

Figure 1. VIP-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/VPAC1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a VPAC1 receptor agonist, VIP. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of VIP (Mean ± SD, n = 2). The EC_{50} of VIP on VPAC1 co-expressing with Gα15 in CHO-K1 cells was 11 nM. The S/B of VIP on VPAC1 co-expressing with Gα15 in CHO-K1 cells was 12.

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. 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. Sreedharan SP *et al.* (1995) Structure, expression, and chromosomal localization of the type I human vasoactive intestinal peptide receptor gene. *Proc Natl Acad Sci* U S A. 92(7):2939-43.
2. Gaudin P *et al.* (1996) Stable expression of the recombinant human VIP1 receptor in clonal Chinese hamster ovary cells: pharmacological, functional and molecular properties. *Eur J Pharmacol.* 302(1-3):207-14.
3. Nicole P *et al.* (2000) Identification of key residues for interaction of vasoactive intestinal peptide with human VPAC1 and VPAC2 receptors and development of a highly selective VPAC1 receptor agonist. Alanine scanning and molecular modeling of the peptide. *J Biol Chem.* 275(31):24003-12.

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