

Human Recombinant Vasoactive Intestinal Polypeptide Receptor Stable Cell Line**Cat. No. M00203****Version 06092014**

I	Introduction	1
II	Background.....	1
III	Representative Data.....	2
IV	Thawing and Subculturing.....	3
V	References	3
	Limited Use License Agreement.....	4

I. INTRODUCTION

Catalog Number: M00203

Cell Line Name: CHO-K1/PAC1/Gα15

Gene Synonyms: PAC1; PACAPR; PACAPRI/FLJ16511; VPAC2; VPCAP2R

Expressed Gene: Genbank Accession Number NM_001118; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10⁶ per vial)

Stability: 16 passages

Application: Functional assay for PAC1 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, 400 µg/ml G418

Mycoplasma Status[§]: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. BACKGROUND

The vasoactive intestinal peptide/pituitary adenylate cyclase activating polypeptide (VPAC) receptors are divided into at least three types: PAC1, VPAC1, and VPAC2. Several splice variants of PAC1 result in proteins that differ at the N-terminus and the third intracellular loop. These variants differ in their affinities for PACAP and abilities to activate Gq and Gs. High level of PAC1 expression is observed in the CNS and the adrenal medulla.

[§]: 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.

860 Centennial Ave., Piscataway, NJ 08854, USAToll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by PACAP in CHO-K1/PAC1/Gα15 and CHO-K1/Gα15 cells

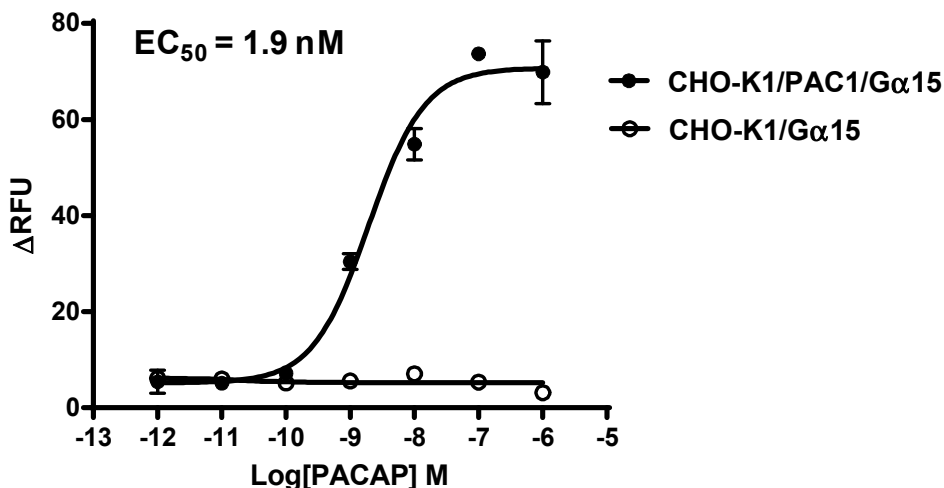


Figure 1. PACAP-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PAC1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a PAC1 receptor agonist, PACAP. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of PACAP (Mean ± SD, n = 2). The EC₅₀ of PACAP on PAC1 co-expressing with Gα15 in CHO-K1 cells was 1.9 nM. The S/B of PACAP on PAC1 co-expressing with Gα15 in CHO-K1 cells was 14.

Notes:

1. EC₅₀ value is calculated with four parameter logistic equation:

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

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.
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.

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6. Grow the cells in incubator with 37°C, 5 %CO₂.
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 %CO₂.

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

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Schmuck, k. (1994) Cloning and functional characterization of the human V1aserotonin receptor .*FEBS Lett*, 342, 85 - 90.
2. Delporte, C. (1995) Pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal peptide stimulate two signaling pathways in CHO cells stably transfected with the selective type I PACAP receptor. *Mol Cell Endocrinol.*, 107, 71 - 76.

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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