

Human Recombinant V1B Vasopressin Receptor Stable Cell Line**Cat. No. M00205****Version 06092014**

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I. INTRODUCTION

Catalog Number: M00205

Cell Line Name: CHO-K1/V1B

Expressed Gene: GenBank Accession Number NM_000707; no expressed tags

Alternate Names: V1BR, arginine vasopressin receptor 1B

Host Cell: CHO-K1

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

Stability: 16 passages

Applications: Functional assays for V1B receptor

Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 400 μ g/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The antidiuretic hormone arginine vasopressin (AVP) receptors are G protein-coupled receptors which consists of at least three types: V1A (vascular/hepatic) and V1B (anterior pituitary) receptors, which act through phosphatidylinositol hydrolysis to mobilize intracellular Ca^{2+} , and V2 (kidney) receptor, which is coupled to adenylate cyclase. V1B receptors are expressed in anterior pituitary where they mediate the release of ACTH. Its peripheral actions, such as antidiuresis, contraction of vascular smooth muscle, and stimulation of hepatic glycogenolysis are well characterized.

§: 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.

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III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by AVP in CHO-K1/V1B and CHO-K1 cells

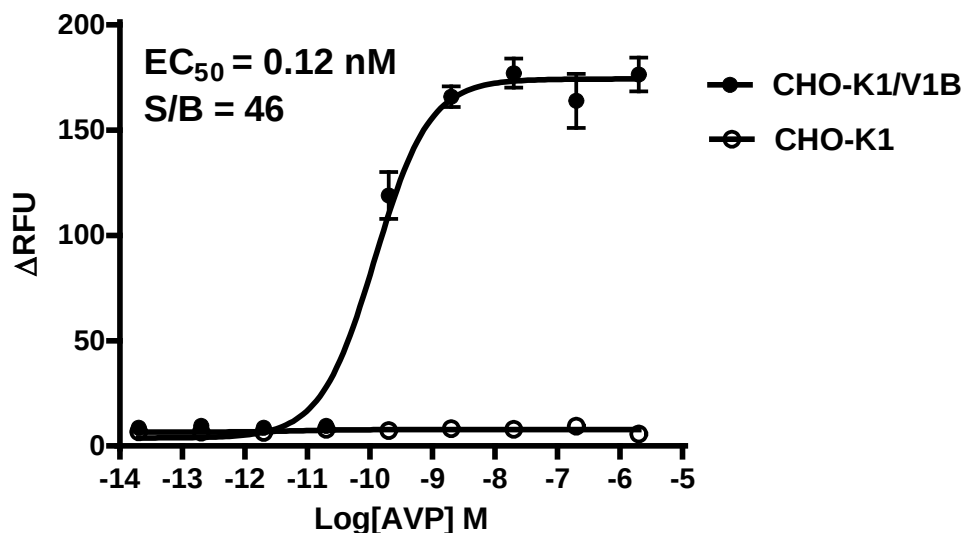


Figure 1. AVP-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/V1B and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a V1B receptor agonist, AVP. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of AVP (Mean $\pm$ SD, n = 2). The EC₅₀ of AVP on V1B in CHO-K1 cells was 0.12 nM. The S/B of AVP on V1B in CHO-K1 cells was 46.

Notes:

- 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.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- 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.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- 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 %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. Sugimoto T (1994) Molecular cloning and functional expression of a cDNA encoding the human V1b vasopressin receptor. *J Biol Chem*, 269, 27088 – 27092
2. de Keyser Y (1994) Cloning and characterization of the human V3 pituitary vasopressin receptor. *FEBS Lett*, 356, 215 - 220

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