Human Recombinant V2 Vasopressin Receptor Stable Cell Line

Cat. No. M00170 **Version 06092014**

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I. INTRODUCTION

Catalog Number: M00170

Cell Line Name: CHO-K1/V2 /Gα15

Gene Synonyms: V2, ADHR, DI1, DIR, DIR3, NDI, V2R

Expressed Gene: Genbank Accession Number NM_000054; 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 V2 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, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Arginine vasopressin (AVP) is a cyclic nonapeptide that acts by binding to a family of vasopressin receptors that includes V1a, V1b, and V2 receptors. In particular, V2 receptors are expressed in kidney where vasopressin exerts its antidiuretic action. V1a and V1b couple to Gq and calcium release, whereas V2 couples to Gs. Mutations in V2 result in X-linked nephrogenic diabetes insipidus, a syndrome in which the kidney is unable to concentrate urine, leading to dehydration and hypernatremia. Conversely, elevated levels of AVP lead to hyponatremia in the syndrome of inappropriate antidiuretic hormone secretion (SIADH), congestive heart failure or cirrhosis, and V2 selective antagonists have been developed to treat these conditions.

§: 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 AVP in CHO-K1/V2/Gα15 and CHO-K1 cells

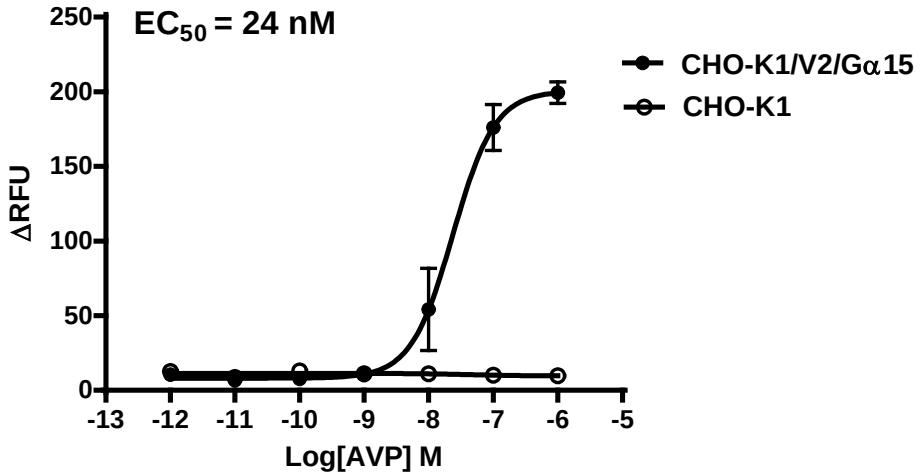

Figure 1. AVP-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/V2/Gα15 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a V2 receptor agonist, AVP. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of AVP (Mean ± SD, n = 2). The EC_{50} of AVP on V2 co-expressing with Gα15 in CHO-K1 cells was 24 nM. The S/B of AVP on V2 co-expressing with Gα15 in CHO-K1 cells was 25.

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 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. COTTE, N., BALESTRE, M.N., PHALIPOU, S., *et al*. (1998) Identification of Residues Responsible for the Selective Binding of Peptide Antagonists and Agonists in the V2 Vasopressin Receptor. *J. Biol. Chem*., 273: 29462-29468.
2. Lolait, S.J., O'Carroll, A.M., *et al*., (1992) Cloning and characterization of a vasopressin V2 receptor and possible link to nephrongenic diabetes insipidus. *Nature* 357: 336-339.
3. Birnbaumer, M., Seibold, A., *et al*., (1992) Molecular cloning of the receptor for human antidiuretic hormone. *Nature* 357: 333-335

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