I. Introduction

Catalog Number: M00445
Cell Line Name: CHO-K1/AT2/Gqι5
Gene Synonyms: AGTR2; AT2; ATR2; MRX88
Expressed Gene: Genbank Accession Number NM_000686; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: Proceeding
Application: Functional assay for AT2 receptor
Freeze Medium: 45% complete growth medium, 45% FBS, 10% DMSO
Complete Growth Medium: Ham’s F12, 10% FBS
Culture Medium: Ham’s F12, 10% FBS, 200 μg/ml Zeocin, 400 μg/ml G418
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. Background

The AT2 receptor couples to G_{a2} or G_{a3}. The activation of the AT2 receptor 1) stimulates Tyr and Ser/Thr phosphatases (MKP-1, PP2A, SHP-1) and protein dephosphorylation, 2) regulates the nitric-oxide-cGMP system and 3) stimulates phospholipase A2, releases arachidonic acid and activates the Na^{+}/HCO_{3} symporter. The expression is developmentally regulated with a higher level at fetal stages. The tissue distributions of AT_{2} receptor are: fetus >> adult; adrenal medulla >> inferior olive, brain >> heart, kidney, ovary, myometrium, endothelial cells, adventitia and pancreas. AGTR2 Inhibits cell growth and stimulates apoptosis in vitro and in vivo. GenScript cloned human AGTR2 cell line co-expressing with Gqι5 in the CHO-K1 cells.
III. Representative Data
Concentration-dependent stimulation of intracellular calcium mobilization by Angiotensin II in CHO-K1/AT2/Gqi5 and CHO-K1/Gqi5 cells

![Graph showing concentration-dependent stimulation of intracellular calcium mobilization by Angiotensin II in CHO-K1/AT2/Gqi5 and CHO-K1/Gqi5 cells.](image)

**Figure 1.** Angiotensin II-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/AT2/Gqi5 and CHO-K1/Gqi5 cells. The cells were loaded with Calcium-4 prior to stimulation with an AT2 receptor agonist, Angiotensin II. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Angiotensin II (Mean ± SD, n = 2). The EC50 of Angiotensin II on AGTR2 co-expressing with Gqi5 in CHO-K1 cells was 7.4 nM. The S/B of Angiotensin II on AGTR2 co-expressing with Gqi5 in CHO-K1 cells was 24.

Notes:
1. EC50 value is calculated with four parameter logistic equation:
   \[ Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^{\text{LogEC}_{50} - X})*\text{HillSlope})} \]
   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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add 200 µg/ml Zeocin and 400 µg/ml G418 to the cells with complete growth medium in the following day.
Subculturing protocol
1. Remove and discard culture medium.
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 to 10 cm dish and observe the cells under an inverted microscope until cell layer is detached (usually within 3 to 5 minutes).
   Note: To avoid clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate detaching.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in the culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References

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