

**Human Recombinant Urotensin 2 Receptor Stable Cell Line**

Cat. No. M00339

Version 06092014

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**I. INTRODUCTION**

Catalog Number: M00339

Cell Line Name: CHO-K1/UT2/Gα15

Gene Synonyms: UTS2R; GPR14; UTR; UTR2

Expressed Gene: Genbank Accession Number NM\_018949; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10<sup>6</sup> per vial)

Stability: 16 passages

Application: Functional assay for UT2 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**

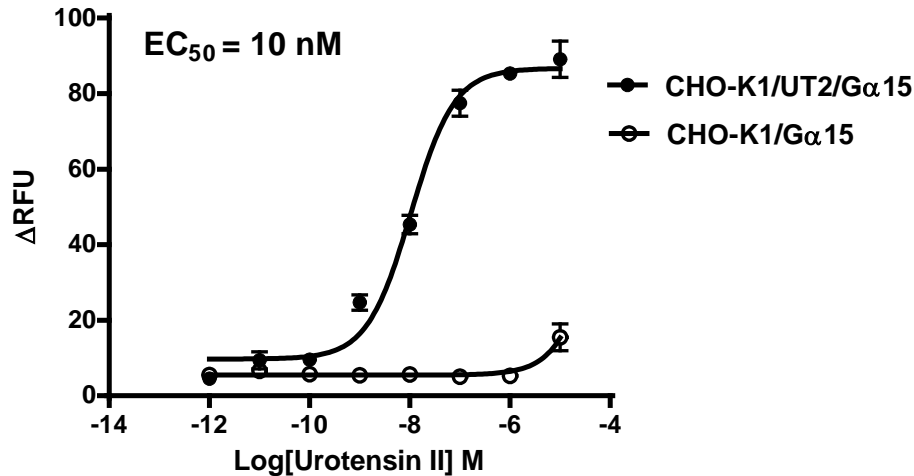
The Urotensin II receptor (UT2), also named UTS2R, GPR14, which is expressed in endothelium, smooth muscle, heart and pancreas. Pharmacological inhibition of urotensin II receptor interactions prevents renal insufficiency following renal artery ligation. .

§: 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 Urotensin II in CHO-K1/UT2/Gα15 and CHO-K1/Gα15 cells



**Figure 1.** Urotensin II-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/UT2/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a UT2 receptor agonist, Urotensin II. 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 Urotensin II (Mean ± SD, n = 2). The EC<sub>50</sub> of Urotensin II on UT2 co-expressing with Gα15 in CHO-K1 cells was 10 nM. The S/B of Urotensin II on UT2 co-expressing with Gα15 in CHO-K1 cells was 10.

**Notes:**

- EC<sub>50</sub> value is calculated with four parameter logistic equation:  

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})}$$
 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.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
- Grow the cells in incubator with 37°C, 5 %CO<sub>2</sub>.

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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<sub>2</sub>.

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

Medium Renewal: Every 2 to 3 days

## V. REFERENCES

1. Ames RS *et al.* (1999) Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. *Nature*. 401(6750):282-6.
2. Clozel M *et al.* (2004) Pharmacology of the urotensin-II receptor antagonist palosuran (ACT-058362; 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulfate salt): first demonstration of a pathophysiological role of the urotensin System. *J Pharmacol Exp Ther*. 311(1):204-12.

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