

---

**Human Recombinant Urotensin 2 Receptor Stable Cell Line****Cat. No. M00339****Version 07302020**

---

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

---

**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

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10<sup>6</sup> per vial)

Stability: More than 16 passages

Application: Functional assay for UT2 receptor (Calcium flux assay)

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen), 400 µg/ml G418 (Cat. #10131-035, Gibco)

Mycoplasma Status: Negative\*

Storage: Liquid nitrogen immediately upon receipt

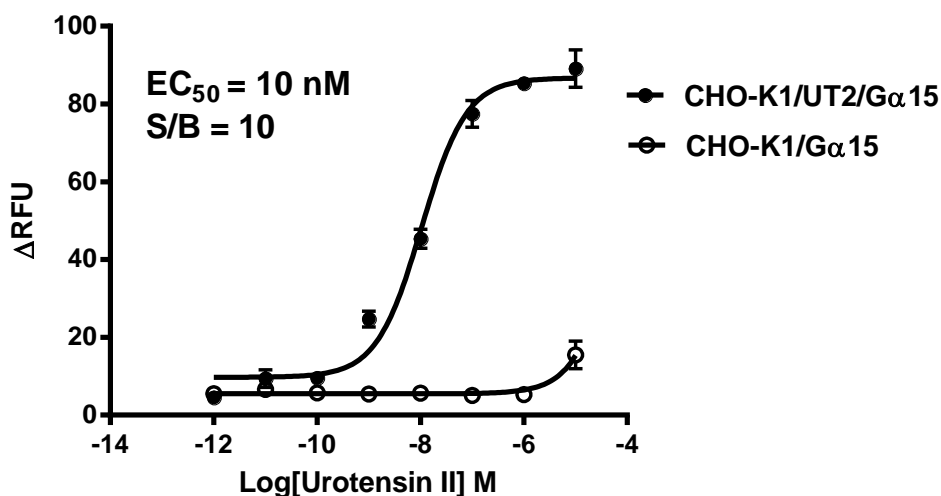
**II. BACKGROUND**

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. .

\* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

### III. REPRESENTATIVE DATA

Calcium mobilization assay:



**Figure 1.** Urotensin II-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/UT2/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist urotensin II. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and 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 this cell was 10 nM.

Notes:

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

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

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.
6. Grow the cells in incubator with 37°C, 5 %CO<sub>2</sub>.
7. Add antibiotic in the following day.

### 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

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.

**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](mailto:product@genscript.com)  
Web: <http://www.genscript.com>  
**For Research Use Only.**

---

## Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

- 1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
- 2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
- 3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 860 Centennial Ave. Piscataway, NJ 08854, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: [marketing@genscript.com](mailto:marketing@genscript.com).