

**Human Recombinant Growth Hormone Releasing Hormone Receptor Stable Cell Line****Cat. No. M00314****Version 05272014**

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**I. INTRODUCTION**

Catalog Number: M00314

Cell Line Name: 293/GHRH/Gα15

Gene Synonyms: GHRHR; GHRFR; GHRHRpsv; GRFR

Expressed Gene: Genbank Accession Number NM\_000823; no expressed tags

Host Cell: 293

Quantity: Two vials of frozen cells ( $3 \times 10^6$  per vial)

Stability: 16 passages

Application: Functional assay for GHRH receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 200 µg/ml Zeocin, 200 µg/ml Hygromycin B, 500 µg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

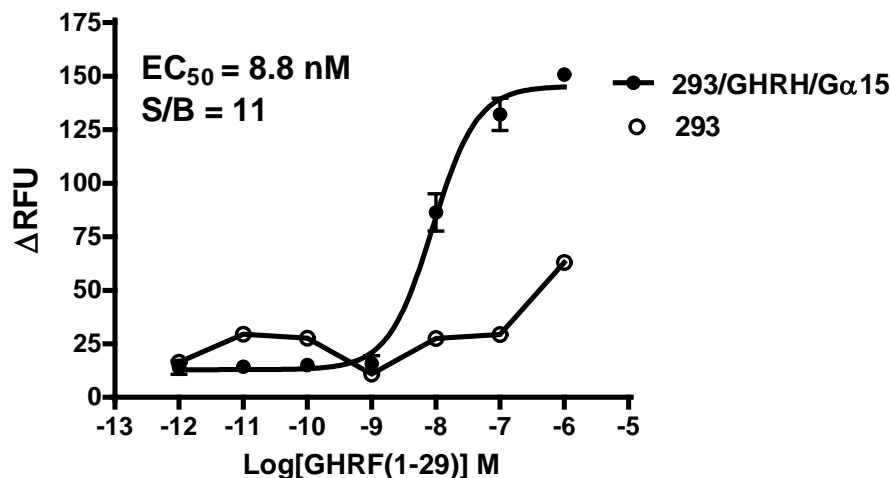
**II. BACKGROUND**

The growth hormone releasing hormone receptor GHRH is Gs-coupled GPCRs expressed in the pituitary and kidney. GHRH receptor stimulates growth hormone gene transcription and release. GenScript's cloned human GHRH-expressing cell line is made in the 293 host.

§: 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 GHRF (1-29) in 293/GHRH/Gα15 and 293 cells



**Figure 1.** GHRF (1-29)-induced concentration-dependent stimulation of intracellular calcium mobilization in 293/GHRH/Gα15 and 293 cells. The cells were loaded with Calcium-4 prior to stimulation with a GHRH receptor agonist, GHRF (1-29). 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 GHRF (1-29) (Mean ± SD, n = 2). The EC<sub>50</sub> of GHRF (1-29) on GHRH co-expressing with Gα15 in 293 cells was 8.8 nM. The S/B of GHRF (1-29) on GHRH co-expressing with Gα15 in 293 cells was 11.

#### Notes:

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

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

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>.
- 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 weekly.

Medium Renewal: Every 2 to 3 days

**V. REFERENCES**

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