

Human Gonadotropin-Releasing Hormone Receptor Cell Line

Technical Manual No. TM0286

Version 20080819



I	Introduction	1
II	Cell Line Information.....	1
III	GnRH Dose Response of HEK/GnRHR/NFAT/ β la Cell Line and Assay Procedure ...	2
IV	References	2
V	Appendix	3
	User Agreement (Label License).....	6

I. Introduction

The gonadotropin-releasing hormone receptor (GnRHR) is a member of the seven-transmembrane G-protein coupled receptor (GPCR) family. It is expressed on the surface of pituitary gonadotrope cells as well as in the lymphocytes, breast, ovaries, and prostate. Following binding of gonadotropin-releasing hormone, the receptor associates with G-proteins that activate a phosphatidylinositol-calcium second messenger system. Activation of the receptor ultimately causes the release of gonadotropic luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Defects in this gene are a cause of hypogonadotropic hypogonadism (HH). This manual describes establishment of a cell line and a protocol of pharmacologically validated human gonadotropin-releasing hormone receptor (Genbank Accession Number: NM_000316).

II. Cell Line Information

- Catalog Number: M00136
- Cell Line Name: HEK/GnRHR/NFAT/ β la
- Description:

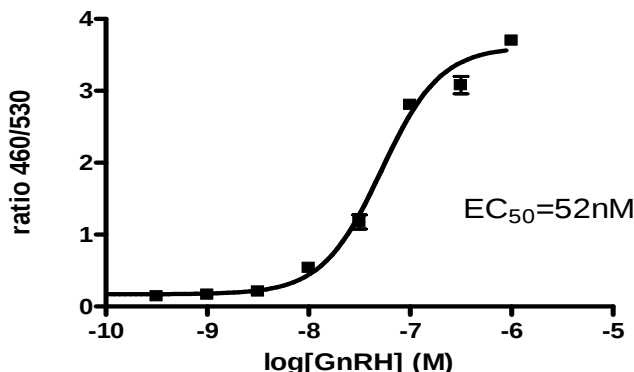
The HUMAN GnRHR is amplified by PCR using a high fidelity enzyme and subcloned into the pcDNA3.1(+) mammalian expression vector. The full-length ORF has been confirmed by sequencing. The GnRHR reporter cell line is created by cotransfection of pcDNA3.1(+)/GnRHR and pNFAT- β la in an Hek293 cell line. The transfected cells are stably selected by 700 μ g/ml G418. Single cell clones with high GnRHR inducibility and low β -lactamase background are isolated using ring cloning. The clones with largest dynamic ranges in β -lactamase are chosen for pharmacological and stability studies.
- Function: Cell based, functional assay for GnRH receptor
- Quantity: 2 vial (2×10^6) frozen cells
- Passage Number Shipped: 2
- Host Cell: HEK293
- Cell Phenotype: Adherent/epithelial
- Mycoplasma: Negative
- Recommended Storage: Liquid nitrogen upon delivery
- Propagation Medium: DMEM, 10% FBS, 700 μ g/ml G418

** For non-clinical research use only. **



III. GnRH Dose Response of HEK/GnRHR/NFAT/ β la Cell Line and Assay Procedure

GnRH Dose Response of HEK/GnRHR/NFAT/ β la Cell Line



1. Seed 25,000 cells per well in growth medium (100 μ l per well) into 96-well tissue culture treated black-wall, clear-bottom plates (Costar #3603) after trypsinization. Prepare some wells with medium alone (no cells) to use for determining plate background.
2. Culture cells in 5% CO₂ at 37°C. Allow cells to reach $\approx$ 90% confluence.
3. 12-24 hours before the assay, replace Growth Medium with 100 μ l/well serum-free DMEM. Be careful not to disturb the cells.
4. Prepare ligand solution in serum-free DMEM (10X).
5. Add 10 μ l of 10X ligand solution to wells for stimulation and 10 μ l of serum-free DMEM per well for non-stimulated control.
6. Incubate cells in 5% CO₂ at 37°C for 5-6 hours.
7. Load cells with 2 μ M CCF4/AM as described in CCF4 Loading Protocol.
8. Incubate the plate at room temperature for 60-120 minutes without shaking.
9. Read with Analyst HT plate reader or similar fluorescence reader.

IV. References

1. Chi Keung Cheng *et al.* Molecular Biology of Gonadotropin-Releasing Hormone (GnRH)-I, GnRH-II, and Their Receptors in Humans. *Endocrine Reviews* 26: 283–306, 2005
2. JIMMY D. NEILL *et al.* Minireview: GnRH and GnRH Receptor Genes in the Human Genome. *Endocrinology* 143: 737–743, 2002
3. Karen L Herbst, Gonadotropin-releasing hormone antagonists. *Current Opinion in Pharmacology* 3:660–666, 2003

** For non-clinical research use only. **



V. Appendix

Cell Culture Conditions

Complete Culture Medium:

DMEM: 90%, FBS: 10%, L-glutamine 2.0 mM, Amp 100 µg/ml, Strep 100 µg/ml, G418 700 µg/ml

Serum-free DMEM:

Same as above but with no FBS, 0.1% BSA

Freezing Medium:

Complete culture medium plus 20% FBS and 10% DMSO

Thawing Cells:

1. Quickly thaw frozen cells in a 37°C water bath, agitating continuously.
2. Using a 1 ml pipette, slowly pipet the cells up and down five times and add, drop by drop, to a 15 ml centrifuge tube containing 5 ml of fresh prewarmed complete DMEM medium. Then centrifuge at 1,000 rpm for five minutes.
3. Discard the supernatant medium and resuspend the cell pellet in 5 ml of fresh prewarmed complete DMEM medium. Transfer cells to a T25 flask and incubate at 37°C with 5% CO₂ until the cells reach >90% confluence. The recovery rate for frozen cells is usually 90% or above.

Subculturing:

When the cells reach confluence, they need split. This cell line is normally split twice weekly at 1:8 to 1:15 dilutions.

1. Carefully aspirate all the media. Gently rinse the cell layer with appropriate amount of 0.2% trypsin-EDTA, and aspirate it off.
2. Wait for about 1-3 minutes. Dislodge the cells by gently tapping the sides of flask or dish.
3. Resuspend cells with appropriate amount of complete DMEM medium, and split cells as desired.

Changing Medium:

This is normally done every other day.

1. Gently aspirate off medium.
2. Transfer fresh warm complete DMEM medium (37°C) into a flask (5 ml for T25 and 10 ml for T75).



Freezing Cells:

1. Repeat steps 1-3 of subculturing section.
2. Centrifuge down the cells at 1,000 rpm for five minutes.
3. Aspirate off the supernatant and resuspend the cells in fresh freezing medium at a density of $2-3 \times 10^6$ cells/ml. Add 1 ml cells per cryogenic vial.
4. Put the cryogenic vial of cells into cryo freezing container. Then transfer the container to a -80°C environment and leave it there overnight.
5. Transfer cryogenic vial into liquid nitrogen (-196°C).

Reagents & Consumables:

1. DMEM: Dulbecco's Modified Eagle Medium powder, high glucose (Gibco BRL, Cat #12100-046)
2. FBS: Fetal Bovine Serum (Hyclone, Cat #CH30160.03)
3. L-Glutamine: 200 mM (Gibco BRL, Cat # 25030-081)
4. Ampicillin: 50 mg/ml (Sigma A-9518)
5. Streptomycin Sulfate: 50 mg/ml (Gibco BRL, Cat # 11860-038)
6. Hygromycin B in PBS, 50 mg/ml (Invitrogen, Cat #10687-010)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)
8. DMSO: dimethyl sulphoxide (Sigma, Cat #D8418)
9. Hepes: Sigma Cat #H-3375
10. CCF4: (Invitrogen, Cat #K1096)
11. GnRH: synthesized
12. Venor[®]GeM Mycoplasma Detection kit: Minerva Biolabs Cat #11-1050
13. 96 Well Plate: Costar, Cat# 3603, Blackwall/clear bottom, Polystyrene, sterilized.

Media and Solutions:

1. PBS (for preparation of 500 ml)

- | | |
|---|----------|
| 1) KCl: | 0.1 g |
| 2) KH_2PO_4 : | 0.1 g |
| 3) NaCl: | 4.0 g |
| 4) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$: | 1.4425 g |

Dissolve the above components in double-distilled water (ddH_2O) and adjust pH to 7.4 with 0.1 N NaOH. Add ddH_2O to the final volume of 500 ml. Autoclave and store at 4°C .

2. Trypsin-EDTA (for preparation of 100 ml)

- | | |
|-------------|--------|
| 1) Trypsin: | 0.25 g |
| 2) 2%EDTA: | 2 ml |
| 3) PBS: | 98 ml |

Dissolve trypsin in 2%EDTA and PBS completely; sterilize the solution by passing through a $0.20 \mu\text{m}$ membrane filter; store at 4°C .

** For non-clinical research use only. **



3. Culture medium (for preparation of 1 L)

- 1) Measure out 950 ml distilled water to dissolve the media components with gentle stirring until the solution becomes clear.
- 2) Add NaHCO_3 3.7 g for high glucose DMEM
- 3) Adjust pH of medium to 0.2-0.3 below the desired final working pH (using 1 N NaOH or 1 N HCL is recommended). Add slowly with stirring.
- 4) Dilute to 1 liter with ddH₂O.
- 5) Sterilize the medium immediately using the method of membrane filtration.
Store at 4°C

4. Ampicillin/Streptomycin 50 mg/ml

Dissolve 1 g Ampicillin or Streptomycin in 20 ml ddH₂O and sterilize the solution by membrane filtration using 0.20 µm filter. Aliquot and store at 4°C for short-term conservation and -20°C for long term conservation.

GenScript Corporation
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: <http://www.genscript.com>

For non-clinical research use only.

**** For non-clinical research use only. ****



User Agreement (Label License) for Human Gonadotropin-Releasing Hormone Receptor Cell Line

Catalog Number: M00136

This is a legal agreement between you (Licensee) and GenScript Corporation governing use of GenScript's Stable GPCR cell line products and protocols provided to licensee. By using this product licensed to the licensee, the licensee is consenting to be legally bound by all of the terms of this user agreement. If you do not agree to all these terms, do not use the products or protocols. All products must be immediately returned to GenScript Corporation for a refund. The licensee will obtain no ownership interest in the product or any use/culture protocols accompanying the product other than through the perpetual limited license granted herein. If the product is licensed through an authorized GenScript distributor, the licensee will be obligated to disclose its identity to GenScript to ensure compliance with this user agreement.

All 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 other uses. The licensee may not sell or otherwise transfer this product or materials made using this product to a third party. The licensee may not use the GenScript products or materials for any commercial purposes. The vendee may transfer information or materials to a scientific collaborator only if such transfer is not for any commercial purpose. "Commercial purposes" means any activity by a vendee of this product for consideration and may include, but is not limited to the following: use of the product or its components in manufacturing; use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; resale of the information or data developed from the product for research and development applications in a service business; or resale of the product or its components, whether or not such product or its components are resold for use in research.

GenScript retains ownership of all patents, copyrights, trademarks, trade secrets and other proprietary rights relating to or residing in the product and every portion thereof. The licensee agrees, (i) that it shall use all products solely in accordance with this agreement and that any such use of products will not violate any applicable law, regulation, judicial order, or injunction; and (ii) that it is not prohibited from receiving the products under U.S. export laws, that it is not a national of a country subject to U.S. trade sanctions, that it will not use the products in a location that is the subject of U.S. trade sanctions that would cover the products, and that, to its knowledge, it is not on the U.S. Department of Commerce's table of deny orders or is otherwise prohibited from obtaining goods of this sort from the United States.

For products that are subject to multiple limited use label licenses, the most restrictive terms apply. GenScript Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by GenScript Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine, or prophylactic product developed in research by the vendee in which this product or its components has been employed, provided that neither this product nor any of its components is used in the manufacture of such product. For information on purchasing a license to this product for purposes other than research, contact Marketing Department, GenScript Corporation, 120 Centennial Avenue, Piscataway, New Jersey 08840. Phone: (732) 885-9188. Fax: (732) 210-0262. Email: marketing@genscript.com

**** For non-clinical research use only. ****