Human Recombinant Gonadotrophin Releasing Hormone Receptor Stable Cell Line

Cat. No. M00426 **Version 07302020**

I. INTRODUCTION

Catalog Number: M00426

Cell Line Name: CHO-K1/GNRHR/Gα15

Gene Synonyms: GNRHR; GNRHR1; GRHR; LHRHR; LRHR

Expressed Gene: Genbank Accession Number NM_000406; no expressed tags

Host Cell: CHO-K1/Gα15

Culture Properties: Adherent

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

Stability: More than 16 passages

Application: Functional assay for GNRHR 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, 400 μg/ml G418 (Cat. #10131-035, Gibco), 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

The gonadotropin-releasing hormone receptor (GNRHR), also known as the luteinizing hormone releasing hormone receptor (LHRHR), 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 lymphocytes, breast, ovary, and prostate. Upon activation, the LHRHr stimulates tyrosine phosphatase and elicits the release of LH from the pituitary. Evidence exists showing the presence of LHRH and its receptor in extrapituitary tissues as well as a role in progression of some cancers.

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

III. REPRESENTATIVE DATA

Calcium mobilization assays:

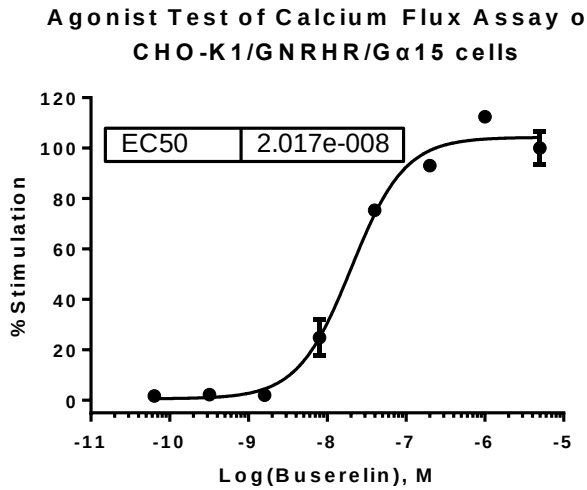

Figure 1. Buserelin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/GNRHR/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist buserelin. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of buserelin (Mean ± SD, n = 2). The EC_{50} of buserelin on this cell was 20.2 nM.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/ (1+10^ ((LogEC_{50}-X)*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_2.
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_2.

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

V. REFERENCES

1. Millar RP, (2005) GnRHs and GnRH receptors. *Anim. Reprod. Sci*. 88(1-2): 5–28.
2. Harrison GS, Wierman ME, Nett TM, *et al*. (2004) Gonadotropin-releasing hormone and its receptor in normal and malignant cells. *Endocr. Relat. Cancer*. 11(4): 725–48.

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
Web: http://www.genscript.com

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