Human Recombinant Corticotropin Releasing Factor Receptor 1 Stable Cell Line

Cat. No. M00343 **Version 05262014**

I. INTRODUCTION

Catalog Number: M00343

Cell Line Name: CHO-K1/CRF1/Gα15

Gene Synonyms: CRF1, CRHR1, CRFR1, CRH1

Expressed Gene: Genbank Accession Number NM_004382; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for CRF1 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, 400 μg/ml G418, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The CRF1 receptor is a Gs-coupled GPCR expressed in the brain and pituitary gland that binds to several neuropeptides, including corticotropin-releasing factor (CRF) and urocortin, and the amphibian peptide sauvagine. CRF plays a predominant role in stress response mediated by the hypothalamic-pituitary-adrenal axis, and alterations in CRF and its receptors CRF1 and CRF2 appear to be linked to depression and anxiety. In comparison to the CRF_2 receptor, the CRF_1 receptor has received considerable attention as a potential therapeutic target for the treatment of stress-related disorders such as adrenocorticotropin hypersecretion, increased colonic motility and exaggerated fear and anxiety-related behavior.

§: 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 Corticotro in CHO-K1/CRF1/Gα15 and CHO-K1/Gα15 cells

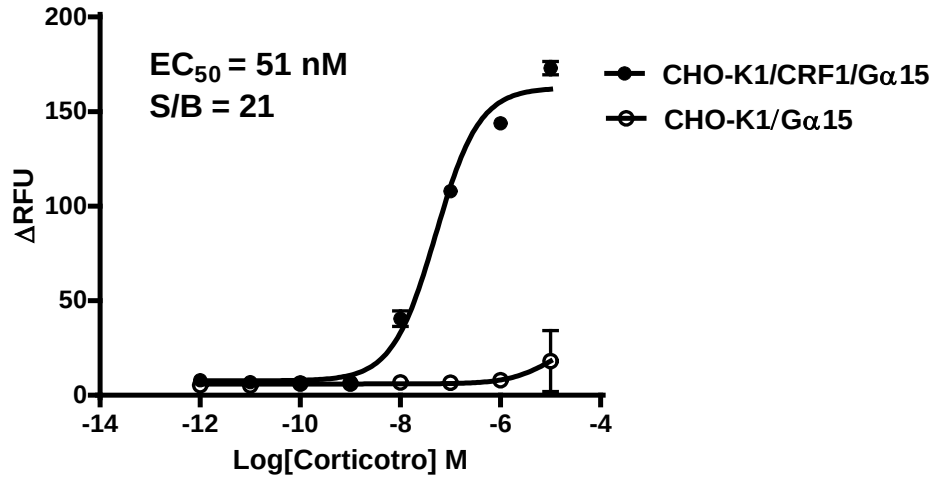

Figure 1. Corticotro-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CRF1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a CRF1 receptor agonist, Corticotro. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Corticotro (Mean ± SD, n = 2). The EC_{50} of Corticotro on CRF1 co-expressing with Gα15 in CHO-K1 cells was 51 nM. The S/B of Corticotro on CRF1 co-expressing with Gα15 in CHO-K1 cells was 21.

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 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. 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_2.

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

V. REFERENCES

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