

Human Recombinant Corticotropin Releasing Factor Receptor CRF2 Stable Cell Line**Cat. No. M00208****Version 05262014**

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

I. INTRODUCTION

Catalog Number: M00208

Cell Line Name: CHO-K1/CRF2/Gα15

Gene Synonyms: CRF2, CRHR2, CRFR2, CRH2

Expressed Gene: Genbank Accession Number NM_001883; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for CRF2 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, 200 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The corticotropin-releasing factor receptor 2 CRF2 is Gs-coupled GPCRs expressed in the brain, blood vessels and intestine that bind to corticotropin-releasing factor (CRF). The CRF is a 41-amino acid peptide that plays an important role in the integration of autonomic, neuroendocrine, and behavioral responses to stress. GenScript's cloned human CRF2-expressing cell line co-expressing Gα15 is made in the CHO-K1 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 Corticotro in CHO-K1/CRF2/Gα15 and CHO-K1 cells

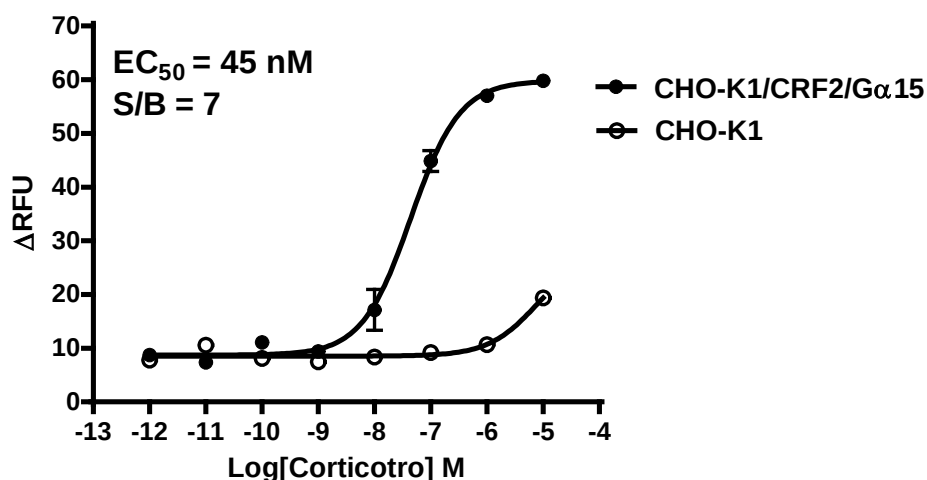


Figure 1. Corticotro-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CRF2/Gα15 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a CRF2 receptor agonist, Corticotro. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Corticotro (Mean ± SD, n = 2). The EC₅₀ of Corticotro on CRF2 co-expressing with Gα15 in CHO-K1 cells was 45 nM. The S/B of Corticotro on CRF2 co-expressing with Gα15 in CHO-K1 cells was 7.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

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

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.
- 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 %CO₂.
- 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 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Lovenberg *et al.*, CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues (1995). *Endocrinology*. 136(9):4139-42
2. Bale, Vale, CRF and CRF receptors: role in stress responsivity and other behaviors (2004) *Annu Rev Pharmacol Toxicol*. 2004;44:525-57. Review

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