Human Recombinant Corticotropin Releasing Factor Receptor CRF2 Stable Cell Line

Technical Manual No. TM0428 **Version 06042010**

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: 2 vial (3×10^6 per vial) frozen cells

Stability: 16 passages

Application: Functional assay for CRF2 receptor

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

Culture Medium: Ham's F12, 10% FBS, 400 μg/ml G418, 200 μg/ml HygromycinB

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.

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III. Representative Data

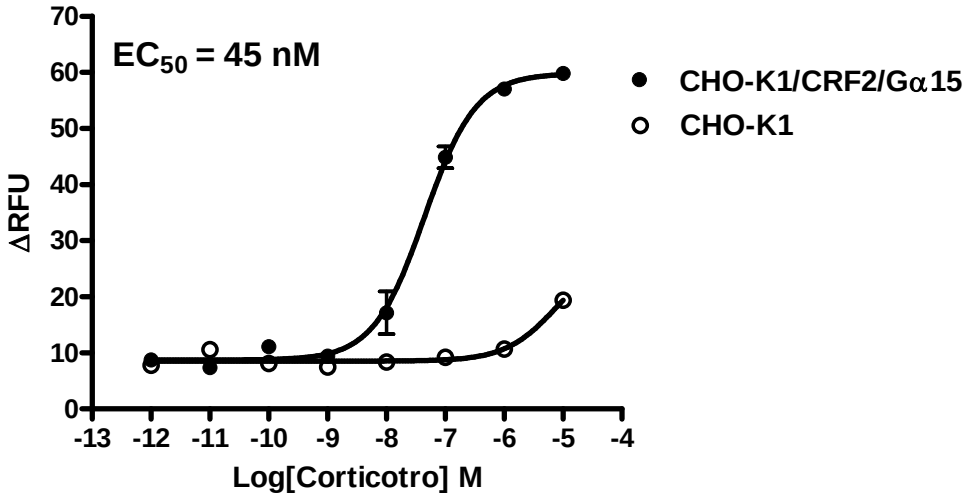

Figure Intracellular calcium response from CHO-K1 cells stably expressing human CRF2 receptor and from untransfected control cells. Cells were loaded with Calcium-4 then stimulated with the indicated concentrations of corticotro. Calcium responses were recorded on a FlexStation plate reader. Data represent the average +/- standard deviation of triplicate determinations.

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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 2 ml of the cell suspension per well in a 6 well-plate.
6. Add Hygromycin B and G418 to concentrations of 200 µg/ml and 400 µg/ml respectively the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in complete growth medium with Hygromycin B and G418 and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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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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