Corticotropin-Releasing Hormone Receptor 1 (CRHR1) ACTOne™ Stable Cell Line

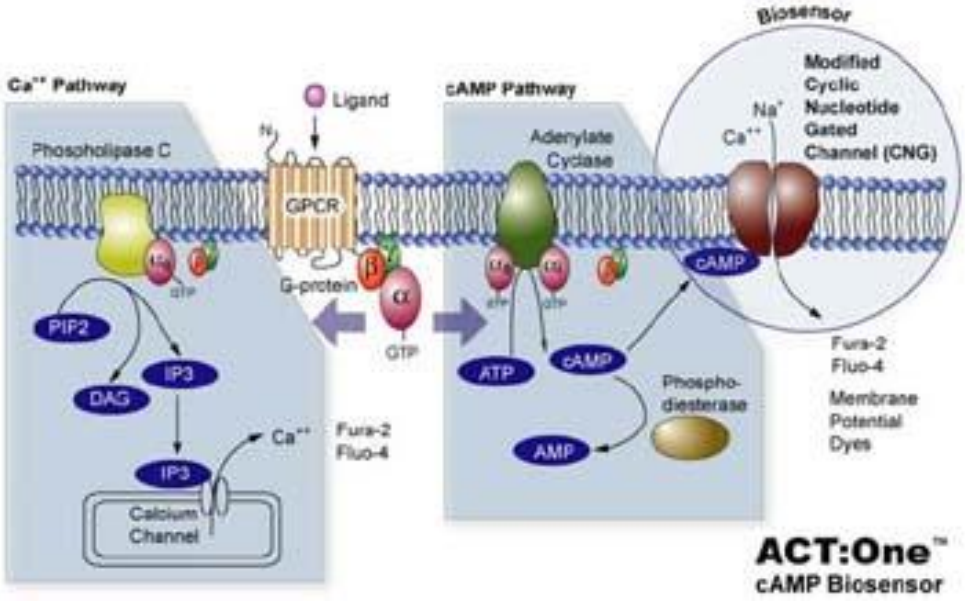
CATALOG NUMBER: CL-01-CRHR1

Introduction

CRHR1 is a G-protein coupled receptor that binds neuropeptides of the corticotropin releasing hormone family that are major regulators of the hypothalamic-pituitary-adrenal pathway. The encoded protein is essential for the activation of signal transduction pathways that regulate diverse physiological processes including stress, reproduction, immune response and obesity.

Description

Human CRHR1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human CRHR1. HEK-293 CNG cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, Cat# CA-C155) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye (Cat# CA-M165). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG cells (originally developed by BD Biosciences by introducing CNG in HEK-293 cells) (Cat# CL-03-PC20)

Gene/Enzyme Introduced

CRHR1 (Genbank Accession No. AAA35718)

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Applications

- cAMP dependent human CRHR1 cell based assay
- cell based high-throughput screening of human CRHR1 inhibitors

Functional Test

- this cell line has been tested positive for CRHR1 specific response
- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

- Stable cells: 1 mL (1 x 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Subculturing Procedure

1. Thaw the frozen cryovial of cells within 1-2 min by gentle agitation in a37 ^oC water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% ethanol and transfer into a 75 cm^2 flask with 20 ml of complete DMEM growth medium.
2. Remove and discard culture medium next day, and then add fresh DMEM complete medium.
3. Monitor cell density daily. Cells should be passaged (1:3) when the culture reaches 90% confluence. Expected cell yield is between 1.5 x 10^5 and 2x 10^5 viable cells/cm^2.
4. Add 2.0 to 3.0 mL of 0.25% (w/v) trypsin-0.53 mM EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 15 to 20 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.
5. Transfer cell suspension to a 15mL centrifuge tube and spin at approximately 250 x *g* for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 4 to 6 x 10^4 viable cells/cm^2 is recommended.
7. Incubate cultures at 37°C (5% CO_2).

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

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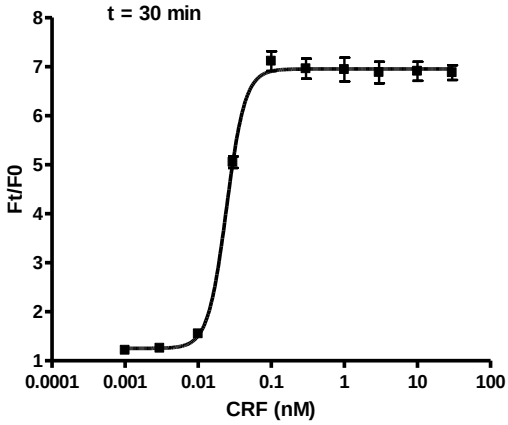
Data Analysis

A



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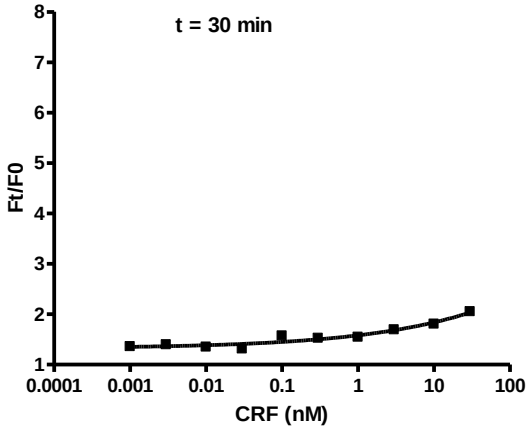

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Figure 1. Response of ACT*One*™ CRHR1 cell line & parental cell line to CRF

ACTOne™ CRHR1 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hour of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of [Nle4, D-Phe7]α-MSH. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of CRFin ACT*One*™ CRHR1 cell line. EC50 = 24 pM in the presence of PDE inhibitor Ro 20-1724, and EC50 = 148 pM in the absence of Ro20-1724 (data not shown).

B. Parental cells do not respond to CRF.

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