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

Catalog Number: CL-01-CRHR1

Product Type: Stable Cell Line

Cell Line Name: ACTOne™ CRHR1

Host Cell Line: HEK-293 CNG

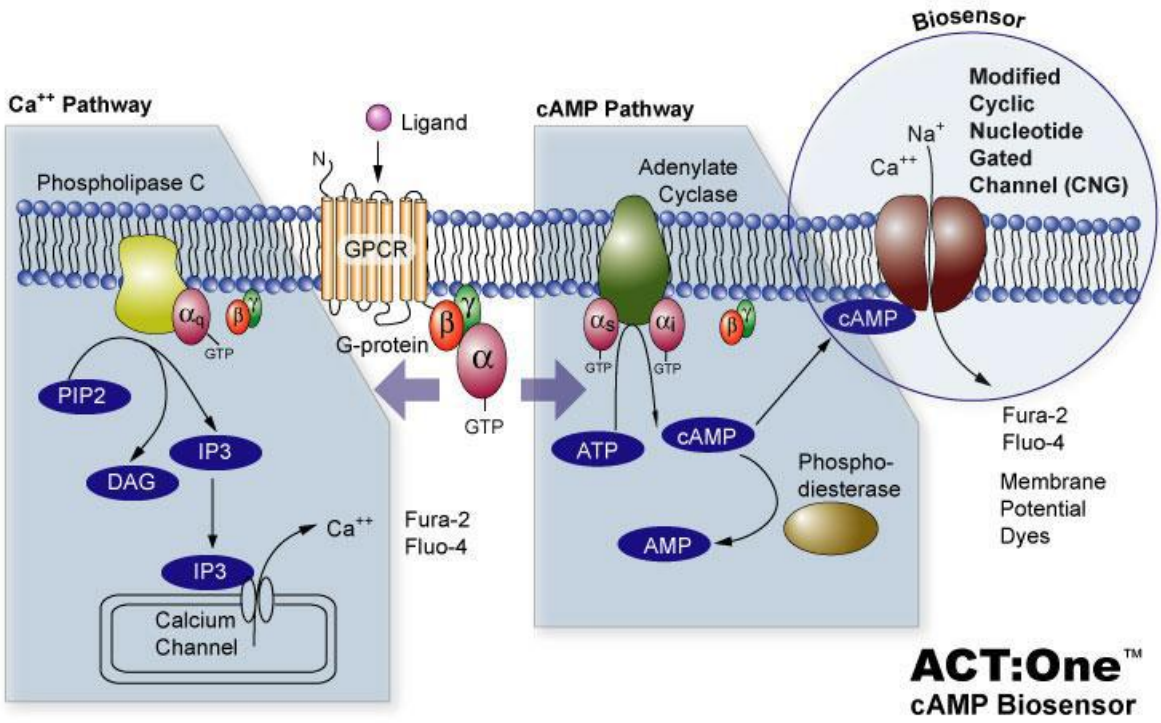
Target Receptor: Human Corticotropin-Releasing Hormone Receptor 1 (CRHR1)

GenBank Accession Number: AAA35718

Product Description

The ACTOne™ CRHR1 stable cell line is a HEK-293 CNG derivative engineered to express recombinant human corticotropin-releasing hormone receptor 1 (CRHR1), a Gs-coupled GPCR that mediates stress hormone signaling via the hypothalamic–pituitary–adrenal (HPA) axis. These cells contain a modified cyclic nucleotide-gated (CNG) channel that opens in response to intracellular cAMP elevation, triggering ion flux and membrane depolarization. This response is quantifiable using fluorescent membrane potential dye (Cat# CA-M165) or calcium-responsive dye (Cat# CA-C155).

The assay supports both endpoint and kinetic measurements of cAMP signaling and is compatible with FLIPR and fluorescence microplate readers



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Applications

- Functional cAMP assays for Gs-coupled CRHR1
- High-throughput screening of CRHR1 modulators
- GPCR pathway analysis in stress biology, neuroendocrinology, and psychiatric research

Functional Validation

- **CRHR1-specific response:** Confirmed
- **Post-thaw viability:** >2.5 million cells/vial on Day 2
- **Receptor activity stability:** Maintained over 10 weeks of continuous passage
- **Mycoplasma status:** Negative (tested lot-specific)

Product Format

- **Volume:** 1 mL
- **Cell Density:** 1x 10^6 cells/mL
- **Cryopreservation Medium:** 70% DMEM, 20% FBS, 10% DMSO

Growth Characteristics

- **Culture Type:** Adherent
- **Growth Medium:** DMEM + 10% FBS, supplemented with 250 µg/mL G418 and 1 µg/mL Puromycin
- **Freezing Medium:** 10% DMSO in complete growth medium

Subculturing Protocol

1. Thaw Cryovial in 37 °C water bath (1-2 min).
2. Decontaminate vial with 70% ethanol.
3. Transfer contents to a 75 cm^2 flask with 20 ml of complete DMEM growth medium.
4. Replace medium after 24 hours.
5. Remove and discard culture medium next day and then add fresh DMEM complete medium.
6. Monitor daily; passage at 90% confluence (1:3 split).
7. Use 0.25% trypsin-0.53 mM EDTA for detachment (15-20 min at 37°C).
8. Centrifuge at 250 x *g* for 5 to 10 min.
9. Resuspend and seed at 4-6 x 10^4 viable cells/cm^2.
10. Incubate at 37°C, 5% CO_2.

Freezing & Storage Instructions

1. Trypsinize cells and resuspend in freezing medium at 2.5 x 10^6 cells/mL.
2. Aliquot 1 mL per cryovial.
3. Freeze overnight at -80°C in a cryo container.
4. Transfer to vapor-phase liquid nitrogen (≤ -130°C) for long-term storage.

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Data Summary

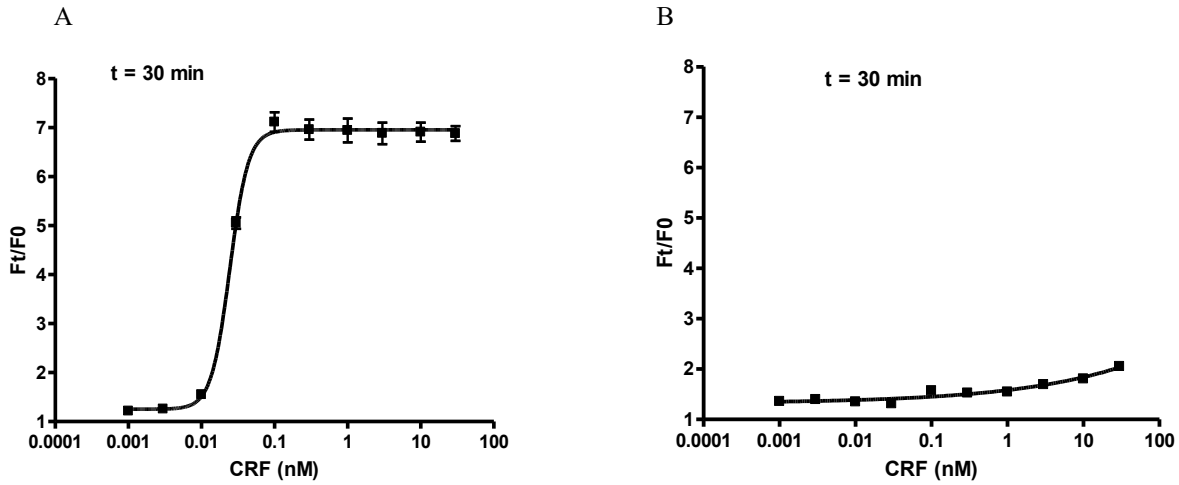

Figure 1. Response of ACT*One*™ CRHR1 cell line & parental HEK-293 CNG cell line to CRF

ACTOne™ CRHR1 cells and parental cells 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 hours 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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