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Calcitonin Receptor-like Receptor (CRLR) ACTOne™ Stable Cell Line

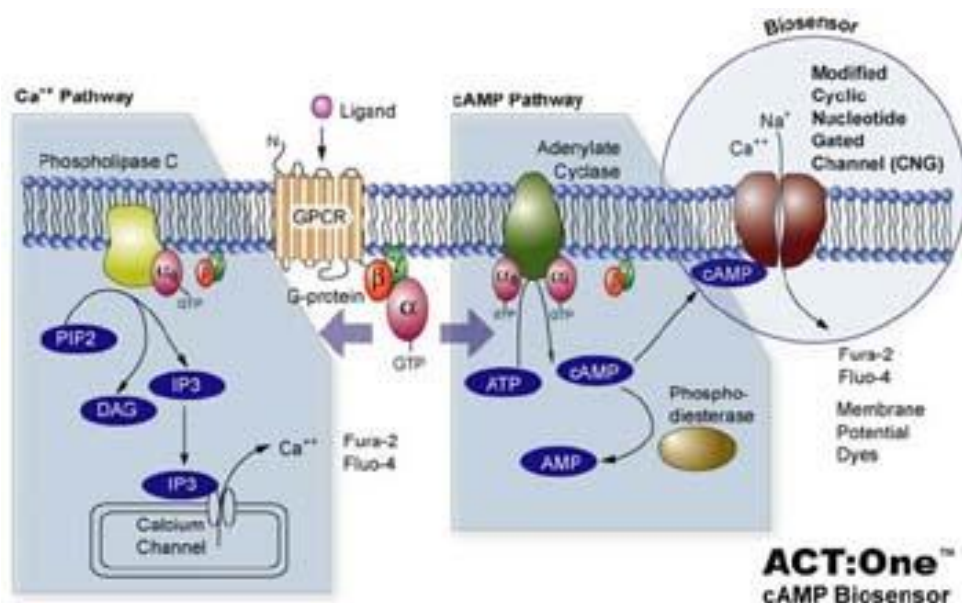
CATALOG NUMBER: CL-01-CRLR

Introduction

CRLR is a G protein-coupled receptor related to the calcitonin receptor. CALCRL is linked to one of three single transmembrane domain receptor activity-modifying proteins (RAMPs) that are essential for functional activity.

Description

Human CRLR ACTOne™ is a HEK293-CNG cell line that expresses recombinant human CRLR. HEK293-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-PC10)

Gene/Enzyme Introduced

CRLR (Genbank Accession No. NP_005786.1)

Applications

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

Functional Test

- this cell line has been tested positive for CRLR 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 (1×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

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

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.

Data Analysis

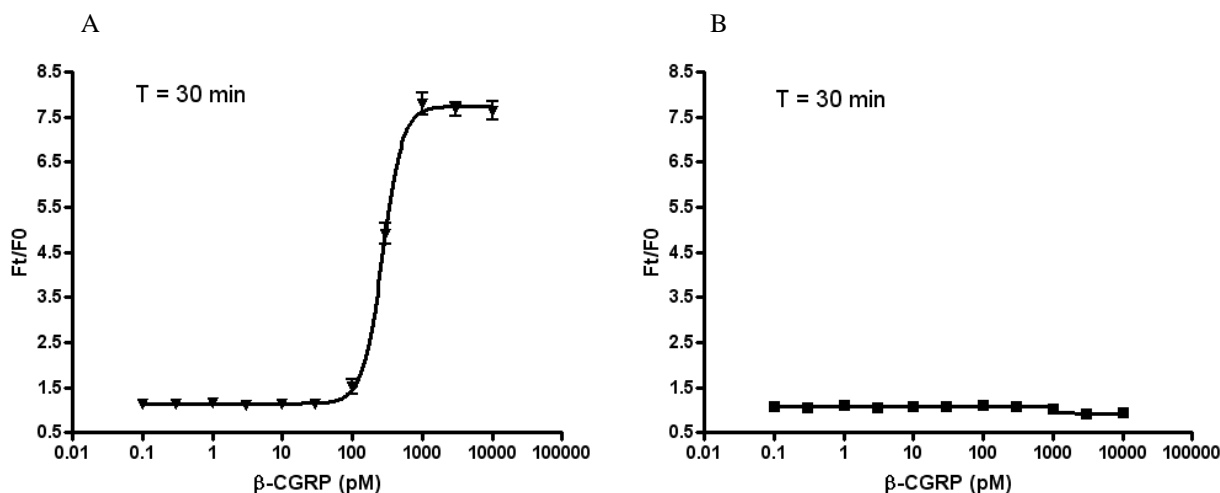


Figure 1. Response of ACTOne™ CRLR cell line & parental cell line to β -CGRP.

ACTOne™ CRLR cells and parental cells (Cat# CL-03-PC10) were plated overnight in 20 μ L culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μ L/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 β -CGRP. Ratios of the two readings (F_t/F_0) are plotted in the figure.

- Dose response curve of β -CGRP in ACTOne™ CRLR cell line. $EC_{50} = 272.3$ pM in the presence of PDE inhibitor Ro 20-1724.
- Parental cells do not respond to β -CGRP.

Notice to Purchaser

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