

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

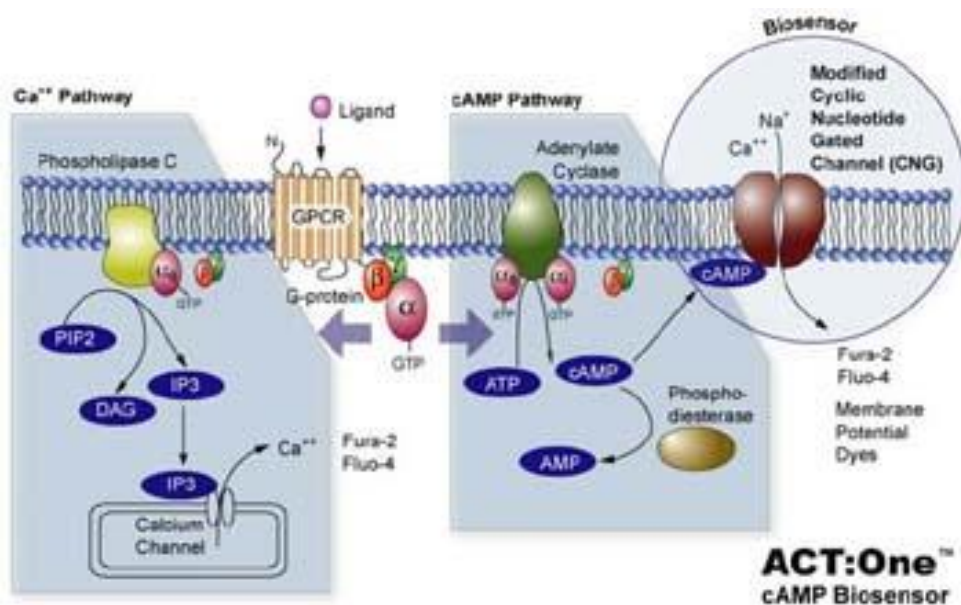
CATALOG NUMBER: CL-01-CRLR

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 x 10⁶ 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

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

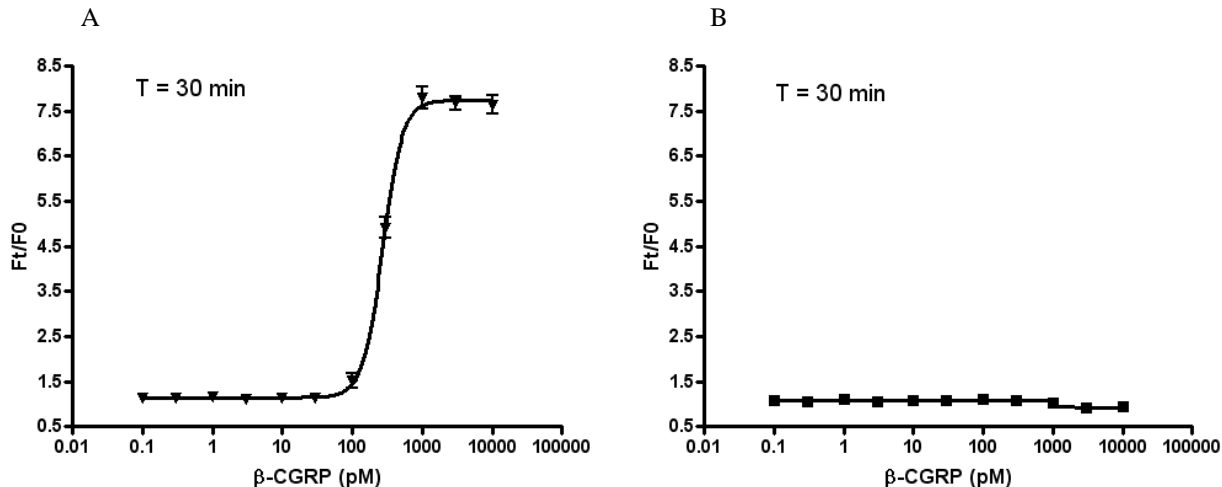


Figure 1. Response of ACTOneTM CRLR cell line & parental cell line to β -CGRP.

ACTOneTM CRLR cells and parental cells (Cat# CL-03-PC10) 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 β -CGRP. Ratios of the two readings (F_t/F_0) are plotted in the figure.

- A. Dose response curve of β -CGRP in ACTOneTM CRLR cell line. EC₅₀ = 272.3 pM in the presence of PDE inhibitor Ro 20-1724.**
- B. Parental cells do not respond to β -CGRP.**

Notice to Purchaser

This cell line is sold with a use license. It may not be transferred to third parties, resold, modified for resale without written approval of eEnzyme LLC. Refer to the license agreements for details on the usage restrictions.