Amylin 3 Receptor (AMY3) ACTOne™ Stable Cell Line

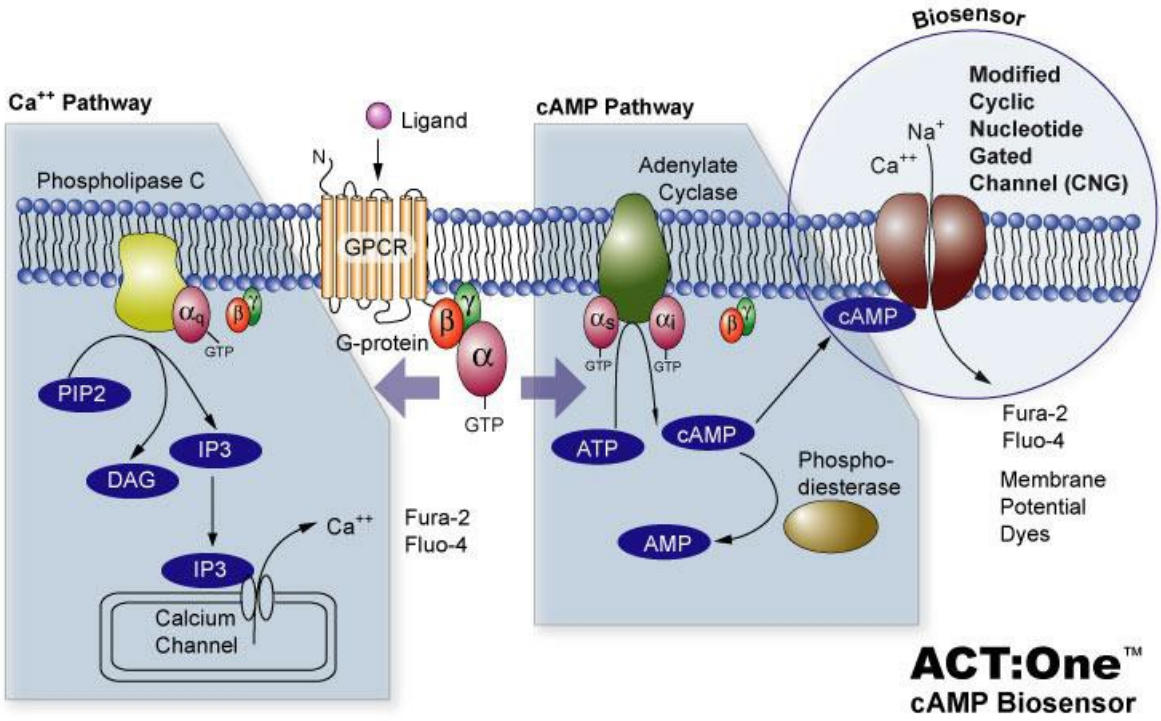
CATALOG NUMBER: CL-01-AMY3

Introduction

Human amylin (AMY) receptors are heterodimers of the CT receptor and receptor activating modifying proteins (RAMPs), which have been implicated in type II diabetes pathologysubtypes. including AMY1 (CT + RAMP1), AMY2 (CT + RAMP2), and AMY3 (CT + RAMP3). RAMP3 is a member of the RAMP family of single-transmembrane-domain proteins, forming multimeric receptor AMY3 with calcitonin receptor (CT).

Description

Human AMY3 Receptor ACTOne™ is a Calcitonin Receptor ACTOne™ stable cell line that expresses recombinant human RAMP3. The modified CNG (Cyclic Nucleotide Gated) channel 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 FDSS, FLIPR, or a fluorescence microplate reader.



Parental Cells

Calcitonin receptor ACTOne™ stable cell line (Cat# CL-01-CALCR)

Gene/Enzyme Introduced

RAMP3 (Genbank Accession No. CAA04474)

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Applications

- cAMP dependent human AMY3 receptor cell based assay
- cell based high-throughput screening of human AMY3 receptor agonists/antagonists

Functional Test

- this cell line has been tested positive for Amylin 3 receptor 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 Amylin 3 receptor 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 a 37°C 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 Example

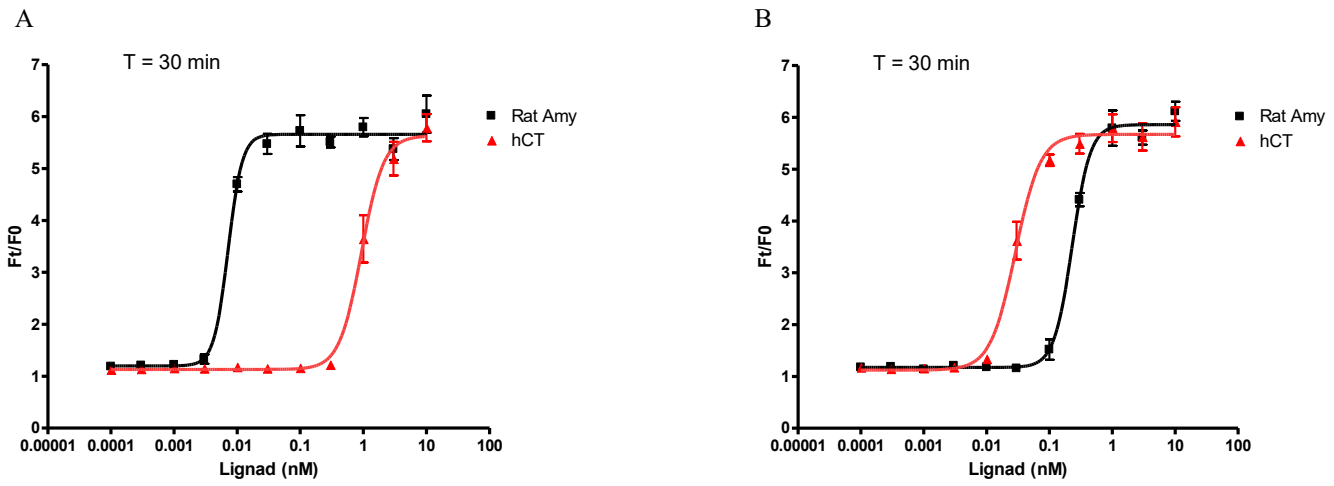

Figure 1. Response of ACT*One*™ AMY3 receptor cell line & parental cell line to amylin and calcitonin
ACTOne™ AMY3 receptor cells and parental cells (Cat# CL-01- CALCR) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/well of 1x 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 rat amylin or human calcitonin (hCT). Ratios of the two readings (F/F0) are plotted in the figure.

A. **Dose response curve of rat amylin and human calcitonin in ACT*One* AMY3 receptor cell line. In the presence of PDE inhibitor Ro20-1724, EC50 = 7.2 pM with rAmy and EC50 = 934 pM with hCT.**
B. **Dose response curve of rat amylin and human calcitonin in Parental cells. In the presence of PDE inhibitor Ro20-1724, EC50 = 230 pM with rAmy and EC50 = 28.7 pM with hCT**

Notice to Purchaser

1.This cell line is to be used for research purposes only. It may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of eEnzyme LLC.
2. Refer to the license agreement for details on the usage restrictions.