

Amylin 3 Receptor (AMY3) ACTOne™ Stable Cell Line

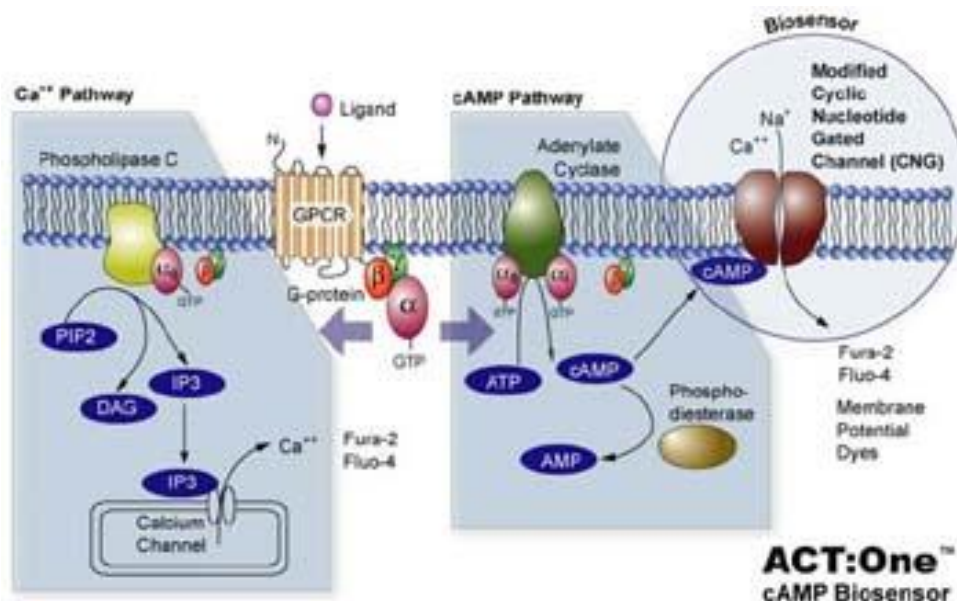
CATALOG NUMBER: CL-01-AMY3

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 pathology subtypes. 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 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 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)

Applications

- cAMP dependent human AMY3 cell based assay
- cell based high-throughput screening of human AMY3 inhibitors

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 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

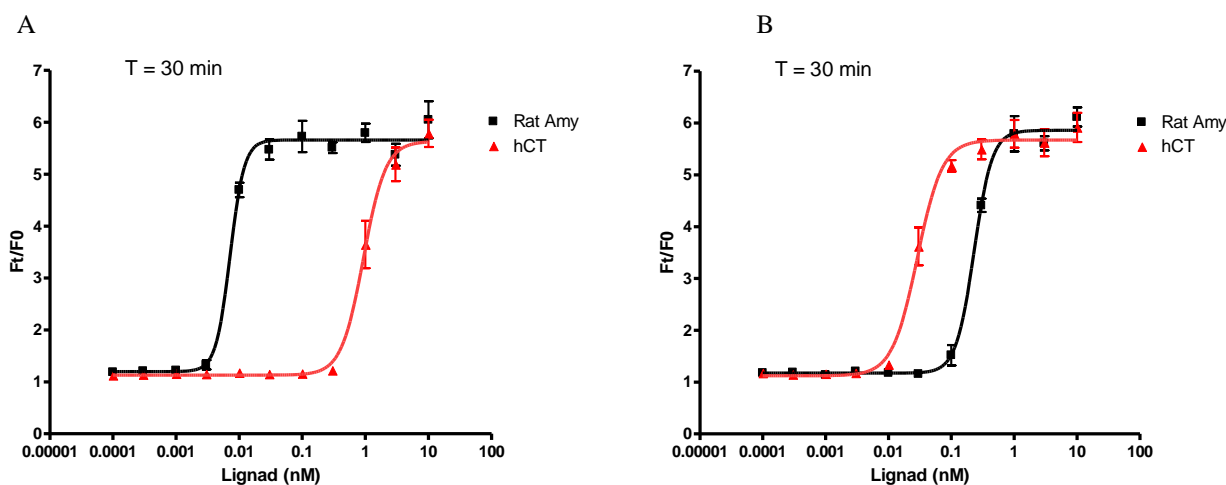


Figure 1. Response of ACTOne™ AMY3 cell line & parental cell line to amylin and calcitonin

ACTOne™ AMY3 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.

- Dose response curve of rat amylin and human calcitonin in ACTOne AMY3 cell line. In the presence of PDE inhibitor Ro20-1724, EC₅₀ = 7.2 pM with rAmy and EC₅₀ = 934 pM with hCT.**
- 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**

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