

DATA SHEET

Catalog #	AG-10200-271
Cell Line Designation	Amylin 3 Receptor cell line
Parental Cell	ACTOne Calcitonin Receptor cells (AG-10200-258)
Gene Introduced	Human receptor activity-modifying protein 3 (RAMP3)
NCBI Accession #	NP_005847

USAGE

- cAMP assay for Gs-coupled human Amylin 3 Receptor (AMY3) or receptor activity-modifying protein 3 (RAMP3).
- ACTOne Calcitonin Receptor cells (AG-10200-258) without transfected RAMP3 are used as a negative control.

QUALITY CONTROL

1. This cell line has been tested negative for *Mycoplasma sp.*
2. This cell line has been tested positive for Amylin 3 Receptor specific response.
3. Surviving rate: More than 2.5 million/vial on the second day after thawing.
4. The receptor specific activity is stable for 10 weeks continuous passage.

CELL CULTURE CONDITION

1. Growth medium: 90% DMEM, 10% FBS, 250 $\mu\text{g/ml}$ G418, 1 $\mu\text{g/ml}$ puromycin and 150 $\mu\text{g/ml}$ hygromycin B
2. Freezing medium: 10% DMSO, 90% complete medium

DATA EXAMPLE

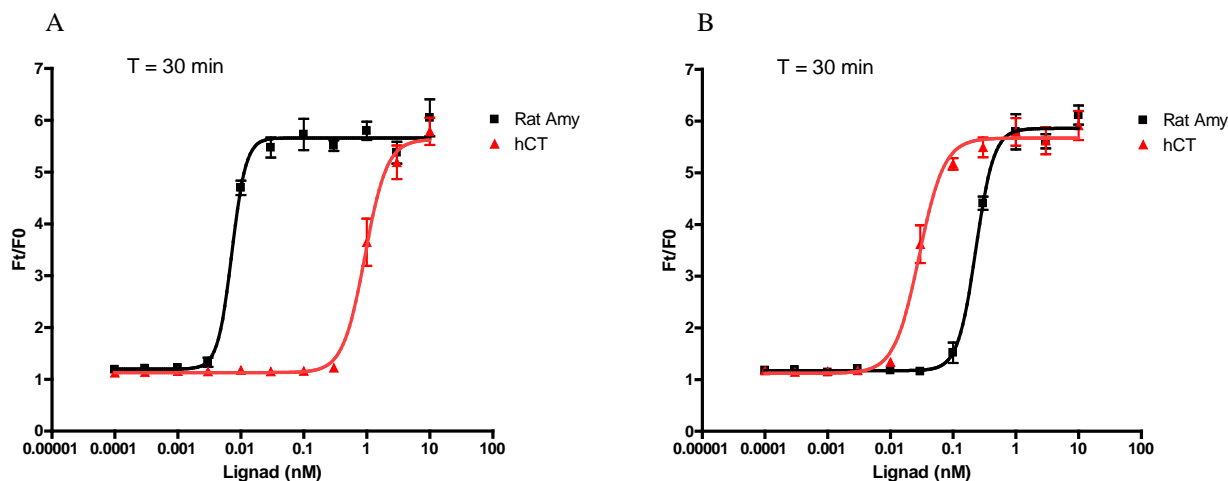


Figure 1. Response of ACTOne AMY3 cell line & parental cell line to amylin and calcitonin.

ACTOne AMY3 cells and parental cells (AG-10200-258) were plated overnight in 20 ml culture medium on a BD Biocoat 384 well plate. The next day, cells were dye-loaded with 20 ml/well of 1X Dye-loading solution (ACTOne Membrane Potential Assay Kit). After 2 hours of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of rat amylin or hCT. Ratios of the two readings (F/F0) are plotted in the figure.

- A. 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.**
- 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**