

Screen Quest™ HEK-CNGC-Amylin 3 Receptor

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 38000 (1 Vial)	Keep in liquid Nitrogen	FLIPR, FDSS, FlexStation, NOVOSTar, ViewLux, IN Cell Analyzer, ArrayScan

Introduction

G protein coupled receptors (GPCR) are one of the largest receptor classes targeted by drug discovery programs. Calcium flux (coupled via Gq pathway) assay is a preferred method in drug discovery for screening GPCR targets. However, over 60% of the known GPCRs signal through adenylyl cyclase activity coupled to cAMP. Most of the existing cAMP assays not only require cell lysis but also lack both temporal and spatial resolution. Screen Quest™ cell lines enable to investigate GPCR that do not conventionally couple through intracellular calcium. Screen Quest™ cell lines are based on a series of G-protein chimeras, including the promiscuous G-protein, G_{α16}, and an exogenous cyclic nucleotide-gated channel (CNGC). The chimeras consist of the alpha subunit of a G_q-protein complex whose 5 carboxy-terminal amino acids have been replaced with those from one of the other G-proteins (either G_{as}, G_{ai}, G_{ao}, or G_{az}). These amino acids are responsible for the coupling of the receptor to its G-protein. Co-expression of these chimeras or CNGC with specific non-G_q-coupled receptors may result in the generation of an intracellular calcium signal upon receptor stimulation.

Screen Quest™ HEK-CNGC-Amylin 3 Receptor (AMY3) cell line is HEK-293 cells stably transfected with both the CNGC and human Amylin 3 Receptor. The constitutively expressed CNGC in the cells responds in real-time to increases or decreases in intracellular cAMP levels by coordinately altering cation flux (e.g., calcium, potassium or sodium). Activation of the AMY3 in these cells by specific ligands can be detected with either a calcium-sensitive fluorescent indicators (such as Calbryte 520 AM, Cal-520 AM, Fluo-8 AM, or Fluo-4 AM and corresponding no wash calcium kits) or an AAT's optimized membrane-potential assay kits. This cell line has been successfully used in drug discovery and screening environments for studying GPCR that do not conventionally couple through intracellular calcium. It has been effectively used with the FLIPR, FDSS Systems.

Handling Procedure for Frozen Cells

1. Cell Density and Storage

The cells are frozen at a density of 2×10^6 cells in 90% fetal bovine serum and 10% (v/v) DMSO. To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. Cells must be stored in liquid nitrogen if not immediately processed upon receipt.

2. Cell Culture Medium

Basal medium: DMEM containing 10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin (Basal medium).

Selection Medium: Basal Medium with 250 µg/mL G418, 1 µg/ml puromycin and 150 µg/ml hygromycin B.

3. Thawing and Seeding Cells

- 1) Prepare Basal Medium. Prepare 37 °C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37 °C water bath. Remove the vial from the water bath as soon as the contents are thawed, and sterilize the exterior of the vial with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- 2) Add vial contents to 25 mL cell culture medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37 °C, 5% CO₂.
- 3) All live cells should be attached after 18-24 hours post-thaw. Change Basal Medium with Selection Medium.

4. Subculture and Propagation

- 1) When cells are approximately 80% confluent, passage the cells.
- 2) Passage the cells 1:10 every 3-4 days, using Trypsin-EDTA to dissociate the cells.
- 3) It is highly recommended that a frozen cell bank be established at low passage number.

Data Analysis

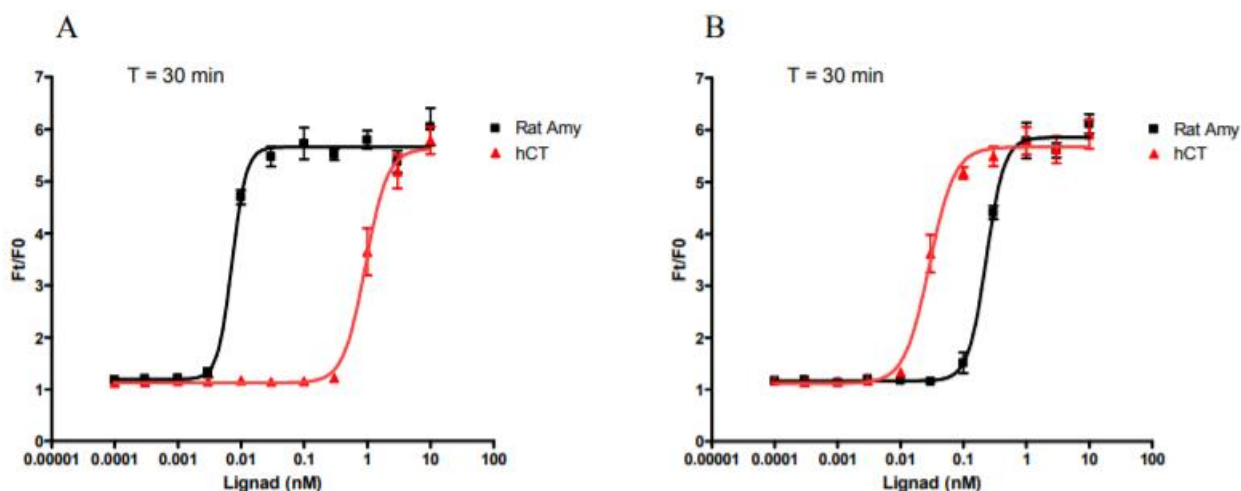


Figure 1. Response of HEK-AMY3 cells and parental cells to amylin and calcitonin.

Both HEK-Aym3 cells and parental cells were plated overnight in 20 μ L/well culture medium in a BD Poly-D-Lysine coated black wall/clear bottom 384-well plate. The cells were incubated with equal volume (20 μ L) of MP dye working solution at room temperature for 2 hours. 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 HEK-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

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