Human Recombinant AMY3 Receptor Stable Cell Line
Cat. No. M00559 **Version 03232016**

I. INTRODUCTION

Catalog Number: M00559

Cell Line Name: CHO-K1/Ga_{15}/AMY3

Gene Synonyms: RAMP3+CALCR

Expressed Gene: Genbank Accession Number NM_005856 +NM_001742; no expressed tags

Host Cell: CHO-K1/$G\alpha_{15}$

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for AMY3 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 200 μg/ml Zeocin, 400 μg/ml G418, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Receptor activity-modifying proteins (RAMPs) are a class of protein which interact with and modulate the activities of several Class B G Protein-Coupled Receptors including the receptors for secretin, calcitonin (CT), glucagon, and vasoactive intestinal peptide (VIP).There are three distinct types of RAMPs, designated RAMP1, RAMP2, and RAMP3, each encoded by a separate gene.

Currently the function of RAMPs is divided into 2 class activities. Association of RAMPs with either the CT or CALCRL proteins forms 6 different receptors from the calcitonin receptor family. When associated with the Calcitonin receptor (CTR) or Calcitonin receptor-like (CALCRL) RAMPs can change the selectivity of the receptor for a specific hormone. In the cases of the other receptors mentioned however, there is no evidence that they can do this, but instead function to regulate trafficking of receptors from the ER / golgi to the membrane.

GenScript's cloned human AMY3–expressing cell line is generated in the CHO-K1/$G\alpha_{15}$ host.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by AM (1-52) in CHO-K1/Ga$_{15}$/AMY3 and CHO-K1/Gα_{15} cells

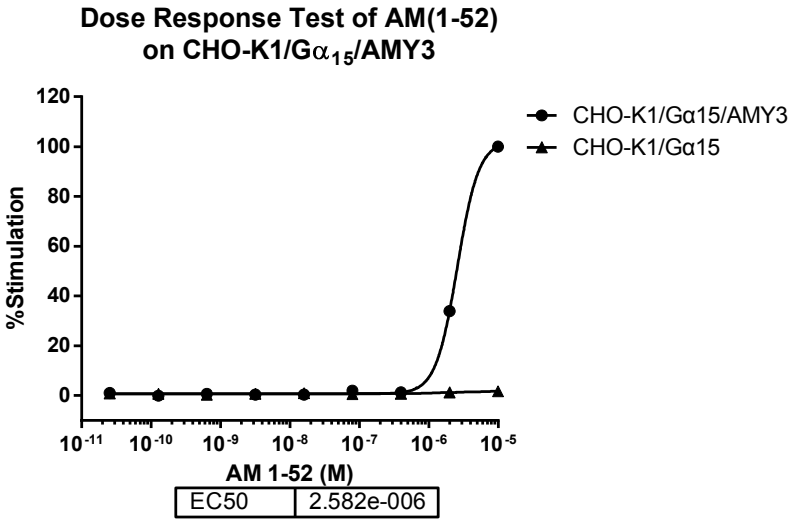

Figure 1. AM (1-52)-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/Ga$_{15}$/AMY3 and CHO-K1/Gα_{15} cells. The cells were loaded with Calcium-4 prior to stimulation with a AMY3 receptor agonist, AM (1-52). The intracellular calcium change was measured by FlexStation. Stimulation% were plotted against the log of the cumulative doses (5-fold dilution) of AM (1-52) (Mean ± SD, n = 4). The EC$_{50}$ of AM (1-52) on AMY3 expressing in CHO-K1/ Gα_{15} cells was 2.587 μM. The S/B of AM (1-52) on AMY3 expressing in CHO-K1/ Gα_{15} cells was 40.

Notes:

1. EC$_{50}$ value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC$_{50}$-X)*HillSlope))
   X is the logarithm of concentration. Y is the response
   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.

6. Grow the cells in incubator with 37°C, 5 %CO_2.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
   Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO_2.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Sexton PM, Morfis M, Tilakaratne N, et.al(2006). Complexing receptor pharmacology: modulation of family B G protein-coupled receptor function by RAMPs. *Ann N Y Acad Sci*. 1070: 90–104.

2. Young A (2005). Amylin: Physiology and Pharmacology; Chapter 3: Receptor pharmacology. *Advances in Pharmacology* 52: 47–65.

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4. McLatchie LM, Fraser NJ, Main MJ, et. al(1998). RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature* 393 (6683): 333–9.

5. Foord SM, Marshall FH (1999). RAMPs: accessory proteins for seven transmembrane domain receptors. *Trends Pharmacol. Sci.* 20 (5): 184–7.

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