
Human Recombinant AMY2 Receptor Stable Cell Line
Cat. No. M00558**Version 08032020**

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I. INTRODUCTION

Catalog Number: M00558

Cell Line Name: CHO-K1/Gα15/AMY2

Gene Synonyms: RAMP2+CALCR

Expressed Gene: Genbank Accession Number NM_005854 +NM_001742; no expressed tags

Host Cell: CHO-K1/Gα15

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10⁶ per vial)

Stability: More than 16 passages

Application: Functional assay for AMY2 receptor

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Complete Culture Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen), 200 µg/ml Zeocin (Cat. #R250-01, Life Technologies)

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. Association of RAMPs with either the CT or CALCR 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.

* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by AM (1-52) in CHO-K1/ Gα15/AMY2 and CHO-K1/Gα15 cells

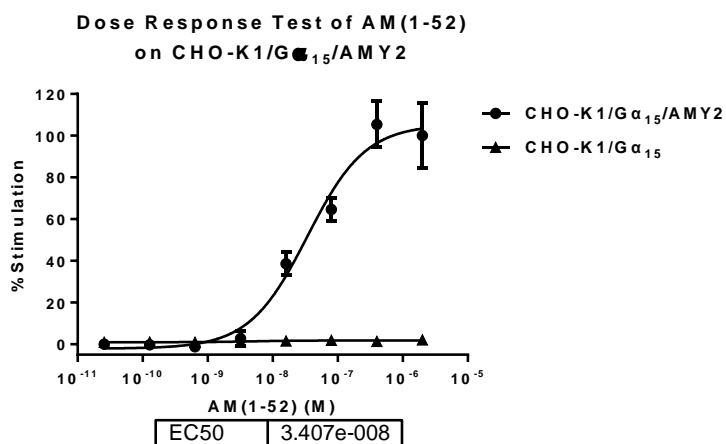


Figure 1. AM (1-52)-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1 /Gα15/AMY2 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an AMY2 receptor agonist, AM (1-52). The intracellular calcium change was measured by FLIPR. Fluorescence signal were normalized and plotted against the log of the cumulative doses (5-fold dilution) of AM (1-52) (Mean ± SD, n = 4). The EC₅₀ of AM (1-52) on this cells was 34 nM.

Notes:

1. EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope})})$$

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. Re-suspend 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 %CO₂.
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 %CO₂.

Subcultivation Ratio: 1:3 to 1:8

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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