Human Recombinant AMY2 Receptor Stable Cell Line
Cat. No. M00558

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
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for AMY2 receptor
Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO
Complete Culture Medium: Ham’s F12, 10% FBS
Culture Medium: Ham’s F12, 10% FBS, 100 μg/ml Hygromycin B, 200 μg/ml Zeocin
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 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.
GenScript’s cloned human AMY2 (RAMP2 + CALCR)–expressing cell line is generated in the CHO-K1/Gα15 host.
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 FlexStation. Stimulation% were 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 AMY2 co-expressing with Gα₁₅ in CHO-K1 cells was 34 nM.

Notes:

1. EC₅₀ value is calculated with four parameter logistic equation:
   \[ Y = Bottom + \frac{Top - Bottom}{1 + 10^{(LogEC_{50} - X) \times HillSlope}} \]
   
   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 5 min, 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 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES
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