Human Recombinant MC3 Melanocortin Receptor Stable Cell Line
Cat. No. M00242 **Version 07272020**

I. INTRODUCTION

Catalog Number: M00242
Cell Line Name: CHO-K1/MC3/Gα15
Gene Synonyms: MC3R; MC3
Expressed Gene: Genbank Accession Number NM_019888; no expressed tags
Host Cell: CHO-K1/Gα15
Culture Properties: Adherent
Quantity: 2 vial (>1×10^6 per vial) frozen cells
Stability: More than 16 passages
Application: Functional assay for MC3 receptor
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 200 μg/ml Zeocin (Cat. #R250-01, Life Technologies), 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

The melanocortin receptor 3, MC3 receptor, is Gs-coupled GPCRs expressed in brain, placental, and gut tissues but not in melanoma cells or in the adrenal gland. MC3 receptor knockouts exhibit a metabolic syndrome. At 4-6 months old, MC3 receptor knockout mice show increased fat mass, reduced lean mass and a higher feed efficiency, with normal metabolic rates. MC3 receptor knockout mice are hyperleptinaemic and males are often mildly hyperinsulinaemic.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by ACTH in CHO-K1/MC3/Gα15 cells

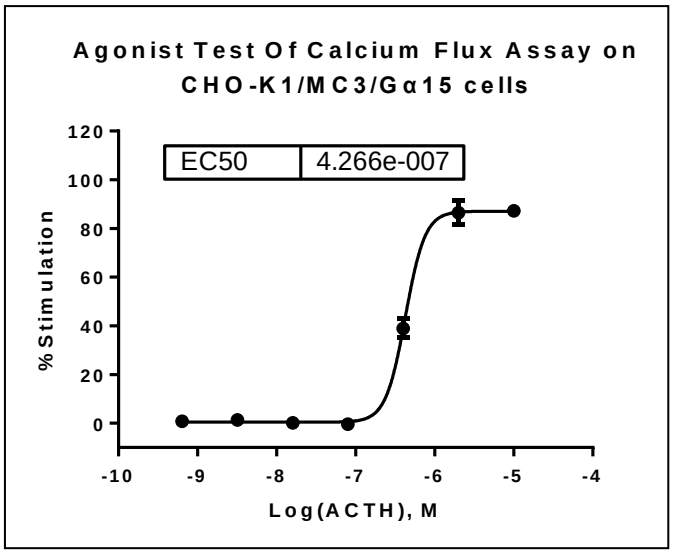

Figure 1. ACTH-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MC3/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with an MC3 receptor agonist, ACTH. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of ACTH (Mean ± SD, n = 2). The EC_{50} of ACTH on this cell was 0.43 μM.

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. Add antibiotic in the following day.

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.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Chen AS *et al,* (2000) Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet.* 26(1):97-102.
2. Gantz I *et al*, (1993) Molecular cloning of a novel melanocortin receptor. *J Biol Chem.* 268(11):8246-50
3. Butler AA *et al*, (2000) A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. *Endocrinology.* 141(9):3518-21.

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