Human Recombinant MC4 Melanocortin Receptor Stable Cell Line
Cat. No. M00272 **Version 07282020**

I. INTRODUCTION

Catalog Number: M00272
Cell Line Name: CHO-K1/MC4/Gα15
Gene Synonyms: MC4, MC4R
Expressed Gene: Genbank Accession Number NM_005912; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for MC4 receptor (calcium flux assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), and 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 family consists of five melanocortin G-Protein-coupled receptors (designated MC1-5R). The melanocortins, α-, β- and γ-melanocyte-stimulating hormones (MSHs), are peptides derived from a precursor protein POMC, and play important roles in energy balance, reproductive function, pigmentation and inflammation. MC4 receptors with high affinities for α-MSH and ACTH are expressed almost exclusively in the CNS and appear to play a prominent role in energy homeostasis.

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

III. REPRESENTATIVE DATA

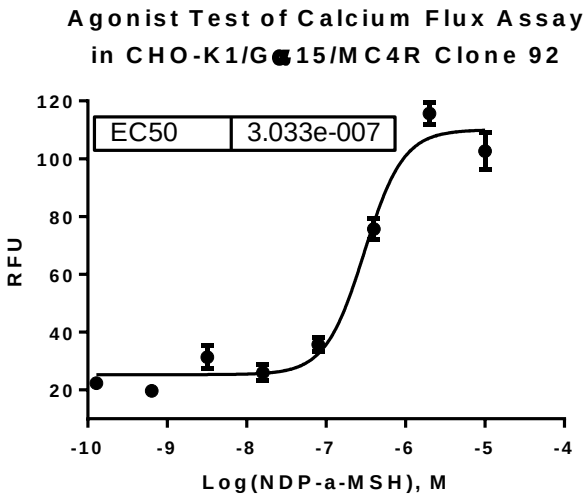

Figure 1. NDP-a-MSH-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/Gα15/MC4 cells. The cells were loaded with Calcium-4 prior to being stimulated with the agonist NDP-a-MSH. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of NDP-a-MSH (Mean ± SD, n = 2). The EC_{50} of NDP-a-MSH on this cell was 0.30 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. Mountjoy, K. G., Kong, P. L., Taylor, J. A., Willard, D. H. and Wilkison, W. O.(2001) Melanocortin receptor-mediated mobilization of intracellular free calcium in HEK293 cells. *Physiol. Genomics*, 5, 11 - 19
2. Yang, Y-K, Dickinson, C., Lae, Y-M., Li, J-Y. and Ira Gantz, I.(2001) Functional properties of an agouti signaling protein variant and binding characteristics of its cognate radioligand. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 281, 1877 - 1886
3. Sawyer, T. K., Sanfilippo, P. J., Hruby, V. J., Engel, M. H., Heward, C. B., Burnett, J. B. and Hadley, M. E.(1980) 4-Norleucine, 7-D-phenylalanine-a-melanocyte-stimulating hormone: A highly potent α-melanotropin with ultralong biological activity. *Proc. Natl. Acad. Sci. U.S.A.*, 77, 5754 - 5758

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