Human Recombinant MC2 Melanocortin Receptor Stable Cell Line
Cat. No. M00336 **Version 07302020**

I. INTRODUCTION

Catalog Number: M00336
Cell Line Name: CHO-K1/MC2/Gα15
Gene Synonyms: MC2R; MC2
Expressed Gene: Genbank Accession Number NM_000529; no expressed tags
Host Cell: CHO-K1/Gα15
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for MC2 receptor (Calcium flux assay, cAMP assay)
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 2, MC2 receptor, is G_s-coupled GPCRs expressed in zona fasciculata of the adrenal cortex placental and stimulates production of cortisol. MC2 is a member of the rhodopsin family of 7-transmembrane and it's also known as the ACTH receptor or corticotropin receptor because it is specific for ACTH alone. Activation of the MC2 receptor initiates a cascade of events affecting multiple steps in corticoid steroidogenesis. Mutations in MC2 may result in familial glucocorticoid deficiency, a group of autosomal recessive disorders characterized by resistance to ACTH.

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

III. REPRESENTATIVE DATA

Calcium mobilization assay:

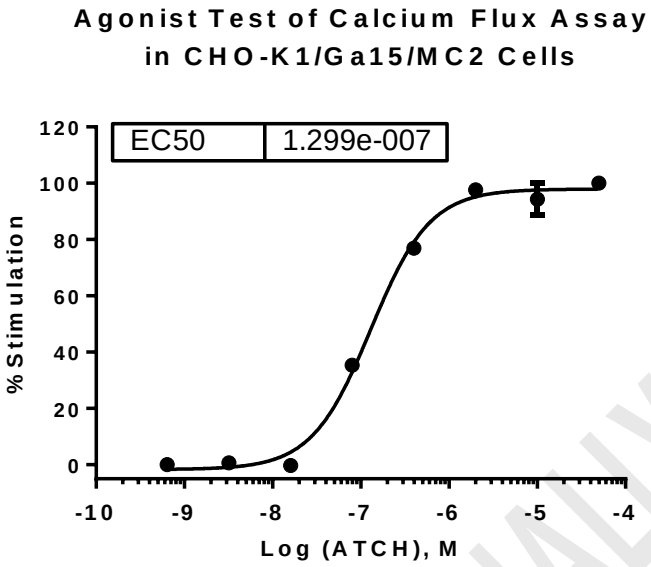

Figure 1. ACTH-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MC2/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist ACTH. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were 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 0.13 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.

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. Cone RD *et al*, (1993) Cloning and functional characterization of a family of receptors for the melanotropic peptides. *Ann. N. Y. Acad. Sci*. 680: 342–63.
2. Tatro JB (1997) Receptor biology of the melanocortins, a family of neuroimmunomodulatory peptides. *Neuroimmunomodulation* 3 (5): 259–84.
3. Gantz I *et al.* (1994). Localization of the genes encoding the melanocortin-2 (adrenocorticotropic hormone) and melanocortin-3 receptors to chromosomes 18p11.2 and 20q13.2-q13.3 by fluorescence in situ hybridization. *Genomics* 18 (1): 166–7.

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