Human Recombinant MC2 Melanocortin Receptor Stable Cell Line

Cat. No. M00336 **Version 05282014**

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
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for MC3 receptor
Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO
Complete Growth Medium: Ham's F12, 10% FBS
Culture Medium: Ham's F12, 10% FBS, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

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.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by ACTH in CHO-K1/MC2/Gα15 and CHO-K1/Gα15 cells

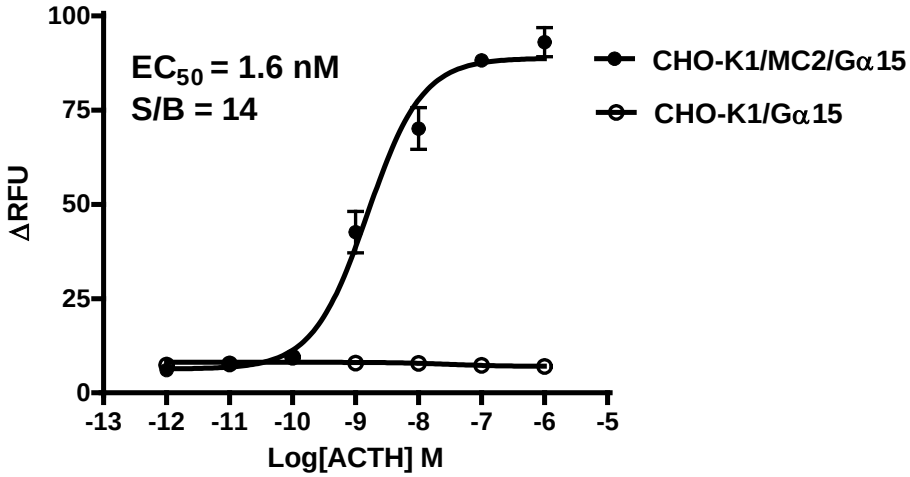

Figure 1. ACTH-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MC2/Gα15 and CHO-K1/Gα15 cells.The cells were loaded with Calcium-4 prior to stimulation with an MC2 receptor agonist, ACTH.The intracellular calcium change was measured by FlexStation.The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of ACTH (Mean ± SD, n = 2).The EC_{50} of ACTH on MC2R co-expressing with Gα15 in CHO-K1 cells was 1.6 nM.The S/B of ACTH on MC2R co-expressing with Gα15 in CHO-K1 cells was 14.

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 weekly.
   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.

GenScript USA Inc,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: http://www.genscript.com

For Research Use Only.

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 120 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.