Human Recombinant Melatonin MT1 Receptor Stable Cell Line

Cat. No. M00424 **Version 05229014**

I. INTRODUCTION

Catalog Number: M00424
Cell Line Name: CHO-K1/MT1/Gα15
Gene Synonyms: MTNR1A; MT1; MEL-1A-R
Expressed Gene: Genbank Accession Number NM_005958; 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 MT1 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

Melatonin binds to two specific G-protein coupled receptors (GPCR), MT1 (MTNR1A/MEL1A) and MT2 (MTNR1B/MEL1B). MT_1 receptors signal via inhibitory G proteins ($G\alpha_i$ and $G\alpha_o$) leading to adenylate cyclase inhibition and possibly inositol phosphate stimulation in recombinant systems. In certain native tissues (e.g. sheep pars tuberalis, rat cerebral and caudal arteries) melatonin responses are presumably mediated through activation of MT_1 receptors. The hypothalamic suprachiasmatic nucleus appears to be involved in circadian rhythm while the hypophysial pars tuberalis may be responsible for the reproductive effects of melatonin.

§: 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 Melatonin in CHO-K1/MT1/Gα15 and CHO-K1/Gα15 cells

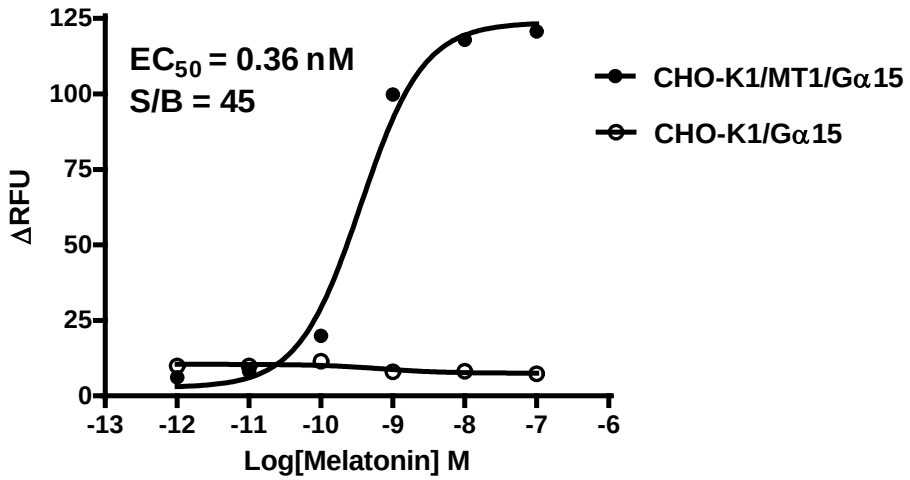

Figure 1. Melatonin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MT1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an MT1 receptor agonist, Melatonin. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Melatonin (Mean ± SD, n = 2). The EC_{50} of Melatonin on MT1 co-expressing with Gα15 in CHO-K1 cells was 0.36 nM. The S/B of Melatonin on MT1 co-expressing with Gα15 in CHO-K1 cells was 45.

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. Slaugenhaupt SA, Roca AL, Liebert CB, *et al.* (1995) Mapping of the gene for the Mel1a-melatonin receptor to human chromosome 4 (MTNR1A) and mouse chromosome 8 (Mtnr1a). *Genomics*. 27(2): 355–7.
2. Reppert SM, Weaver DR, Ebisawa T, (1994) Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron*. 13(5): 1177–85.
3. Witt-Enderby PA, Masana MI, Dubocovich ML, (1998) Physiological exposure to melatonin supersensitizes the cyclic adenosine 3',5'-monophosphate-dependent signal transduction cascade in Chinese hamster ovary cells expressing the human mt1 melatonin receptor. *Endocrinology*. 139(7): 3064–71.

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