Human Recombinant Melatonin MT2 Receptor Stable Cell Line

Cat. No. M00312 **Version 05292014**

I. INTRODUCTION

Catalog Number: M00312
Cell Line Name: CHO-K1/MT2/Gα15
Gene Synonyms: MTNR1B
Expressed Gene: Genbank Accession Number NM_005959; 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 MT2 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, 400 μg/ml G418, 100 μg/ml Hygromycin B
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Melatonin is a neurohormone that plays a key role in the synchronisation of circadian and seasonal functions with cyclic environmental variations. In mammals, two melatonin receptors, MT1 and MT2, have been cloned. Activation of MT2 melatonin receptors phase shift circadian rhythms of neuronal firing in the suprachiasmatic nucleus, inhibit dopamine release in retina, induce vasodilation and inhibition of leukocyte rolling in arterial beds, and enhance immune responses.

§: 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/MT2/Gα15 and CHO-K1 cells

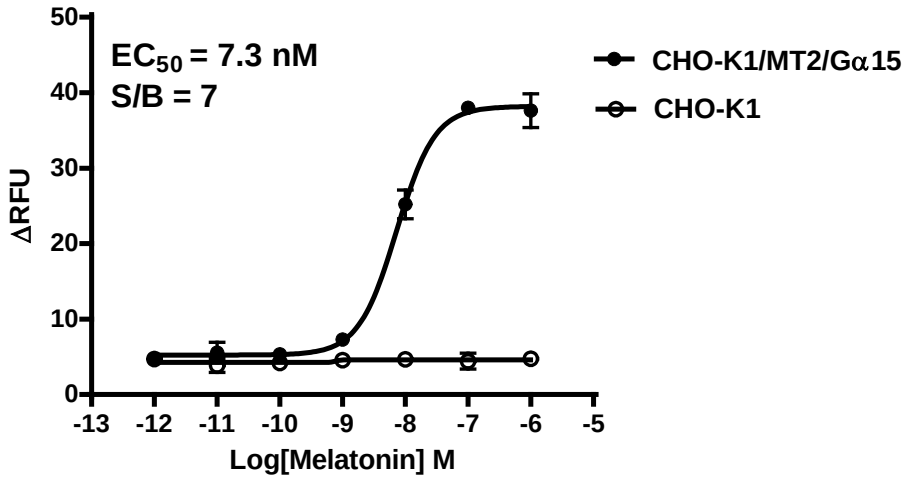

Figure 1. Melatonin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MT2/Gα15 and CHO-K1. The cells were loaded with Calcium-4 prior to stimulation with a MT2 receptor agonist, Melatonin. The intracellular calcium change was measured by FlexStation. The relative 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 MT2 co-expressing with Gα15 in CHO-K1 cells was 7.3 nM. The S/B of Melatonin on MT2 co-expressing with Gα15 in CHO-K1 cells was 7.

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. Schuster C. (2007) Sites and mechanisms of action of melatonin in mammals: the MT1 and MT2 receptors. *J. Soc. Biol.* 201(1):85-96
2. Jockers R, *et al*. (2008) Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *Br. J. Pharmacol.* 154(6):1182-1195.
3. Fisher SP, *et al*. (2009) Sleep-promoting action of IIK7, a selective MT2 melatonin receptor agonist in the rat. *Neurosci. Lett.* 457(2):93-96.

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