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## Human Recombinant Melatonin MT2 Receptor Stable Cell Line

Cat. No. M00312

Version 07292020

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### 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

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10<sup>6</sup> per vial)

Stability: More than 16 passages

Application: Functional assay for MT2 receptor

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, 400 µg/ml G418 (Cat. #10131-035, Gibco), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative\*

Storage: Liquid nitrogen immediately upon receipt

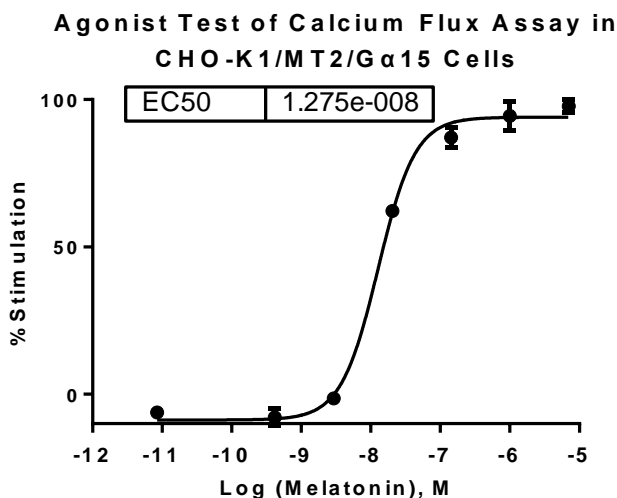
### II. BACKGROUND

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.

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

### III. REPRESENTATIVE DATA

#### Concentration-dependent stimulation of intracellular calcium mobilization by Melatonin in CHO-K1/MT2/Gα15 cells



**Figure 1.** Melatonin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/MT2/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with a MT2 receptor agonist melatonin. The intracellular calcium change was measured by FLIPR<sup>TETRA</sup>. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses of melatonin (Mean ± SD, n = 2). The EC<sub>50</sub> of melatonin on this cell was 12.75 nM.

#### Notes:

- EC<sub>50</sub> value is calculated with four parameter logistic equation:  

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$
 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

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- 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.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
- Grow the cells in incubator with 37°C, 5 %CO<sub>2</sub>.
- 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<sub>2</sub>.

Subcultivation Ratio: 1:3 to 1:8

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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