Human Recombinant Melatonin MT2 Receptor Stable Cell Line

Technical Manual No. TM0449 **Version 06042010**

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: 2 vial (3×10^6 per vial) frozen cells

Stability: 16 passages

Application: Functional assay for MT2 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Culture 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.

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

III. Representative Data

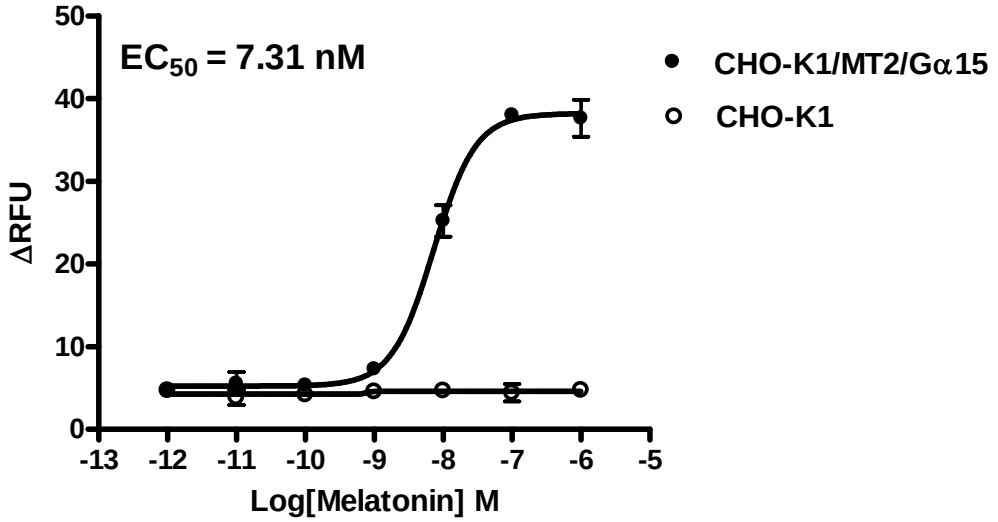

Figure Intracellular calcium response from CHO-K1 cells stably expressing human MT2 receptor and from untransfected control cells. Cells were loaded with Calcium-4 then stimulated with the indicated concentrations of Melatonin. Calcium responses were recorded on a FlexStation plate reader. Data represent the average +/- standard deviation of triplicate determinations.

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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 2 ml of the cell suspension per well in a 6 well-plate.
6. Add Hygromycin B and G418 to concentrations of 100 µg/ml and 400 µg/ml respectively the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in complete growth medium with Hygromycin B and G418 and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

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.

GenScript USA Inc.
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@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.

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com