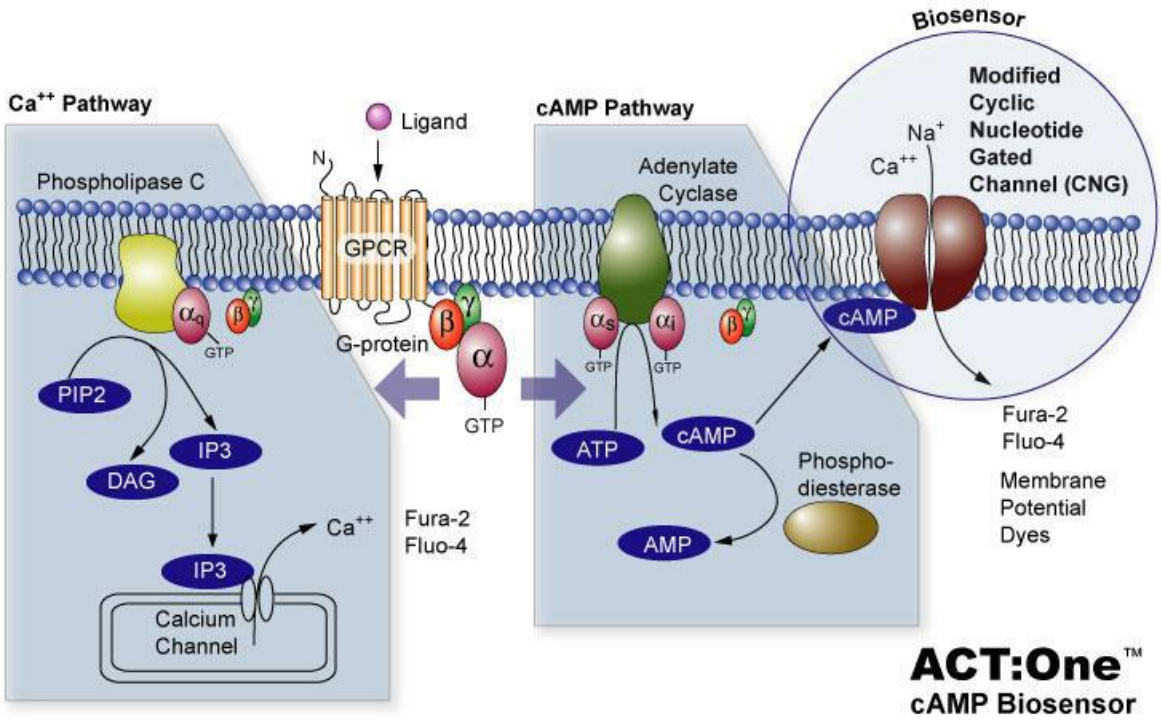
ACTOne™ Human Melatonin Receptor 1B (MT2) Stable Cell Line

Catalog Number: CL-01-MT2
Product Type: Stable Cell Line
Cell Line Name: ACTOne™ MT2
Host Cell Line: HEK-293 CNG
Target Receptor: Human Melatonin Receptor 1B (MT2)
Other Names: MTNR1B; Melatonin Receptor 1B; Mel1b Receptor; MT2 Receptor
GenBank Accession Number: NM_005959

Product Description

The ACTOne™ MT2 stable cell line is a HEK-293 CNG derivative engineered to express recombinant human melatonin receptor 1B (MT2/MTNR1B). MT2 is primarily coupled to Gi/o proteins; receptor activation inhibits adenylyl cyclase and reduces intracellular cAMP. In the ACTOne™ assay, changes in cAMP are detected through a modified cyclic nucleotide-gated (CNG) channel using fluorescent membrane potential dye (Cat# CA-M165) or calcium-responsive dye (Cat# CA-C155).

For Gi/o-coupled receptor assays, intracellular cAMP is first elevated using isoproterenol, and melatonin-dependent inhibition is measured in the presence of a phosphodiesterase inhibitor. The assay supports both endpoint and kinetic measurements and is compatible with FLIPR, FlexStation, FDSS, and fluorescence microplate readers.



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Applications

- Functional cAMP assays for Gi/o-coupled MT2 signaling
- High-throughput screening of MT2 agonists and antagonists
- Melatonin receptor pharmacology, circadian signaling, sleep, and metabolic research

Functional Validation

- MT2-specific response: Confirmed
- **Post-thaw viability:** >2.5 million cells/vial on Day 2
- Reference agonist response: Melatonin EC50 = 0.45 nM under the stated assay conditions
- **Mycoplasma status:** Negative (tested lot-specific)

Product Format

- **Volume:** 1 mL
- **Cell Density:** 2.5 x 10^6 cells/mL
- **Cryopreservation Medium:** 90% FBS, 10% DMSO

Growth Characteristics

- **Culture Type:** Adherent
- **Growth Medium:** DMEM + 10% FBS, supplemented with 250 µg/mL G418 and 1 µg/mL Puromycin
- **Freezing Medium:** 90% FBS, 10% DMSO

Subculturing Protocol

1. Thaw Cryovial in 37 °C water bath (1-2 min).
2. Decontaminate vial with 70% ethanol.
3. Transfer contents to a T25 flask or 10 cm culture dish containing 9 mL of pre-warmed complete growth medium.
4. Replace the medium approximately 4 hours after plating to remove residual DMSO.
5. Allow cells to recover for 1-3 days and expand when they reach 80-90% confluence.
6. Monitor daily; do not allow cells to exceed 90% confluence. For routine passage, split at a ratio of 1:4 to 1:10.
7. Wash once with calcium- and magnesium-free DPBS and detach with 1X trypsin-EDTA for approximately 5 minutes at room temperature.
8. Centrifuge at approximately 200 x g for 5 minutes.
9. Resuspend in complete growth medium and seed at a density appropriate for the selected culture vessel.
10. Incubate at 37°C, 5% CO_2.

Freezing & Storage Instructions

1. Trypsinize cells and resuspend in freezing medium at 2.5 x 10^6 cells/mL.
2. Aliquot 1 mL per cryovial.
3. Freeze overnight at -80°C in a controlled-rate cryo container.
4. Transfer to vapor-phase liquid nitrogen (≤ -130°C) for long-term storage.

Data Summary

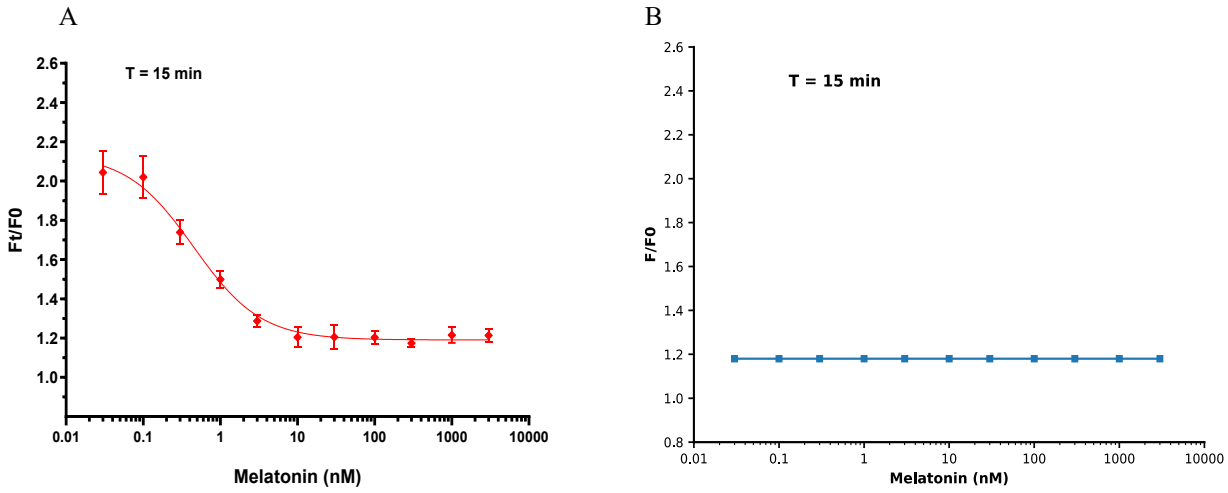

Figure 1. Response of ACT*One*™ MT2 cell line & parental cell line to melatonin.

ACTOne™ MT2 cells and parental HEK-293 CNG cells were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hours of incubation at room temperature, two readings were obtained prior to and 10 min after the addition of melatonin. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of melatonin in ACT*One*™ MT2 cell line. EC50 = 0.45 nM in the presence 25 µM of PDE inhibitor Ro 20-1724 and 300 nM of β-adrenoceptor agonist isoproterenol.

B. Parental HEK-293 CNG cells do not respond to melatonin.

Notice to Purchaser

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