

Melanocortin 1 Receptor (MC1R) ACTOne™ Stable Cell Line

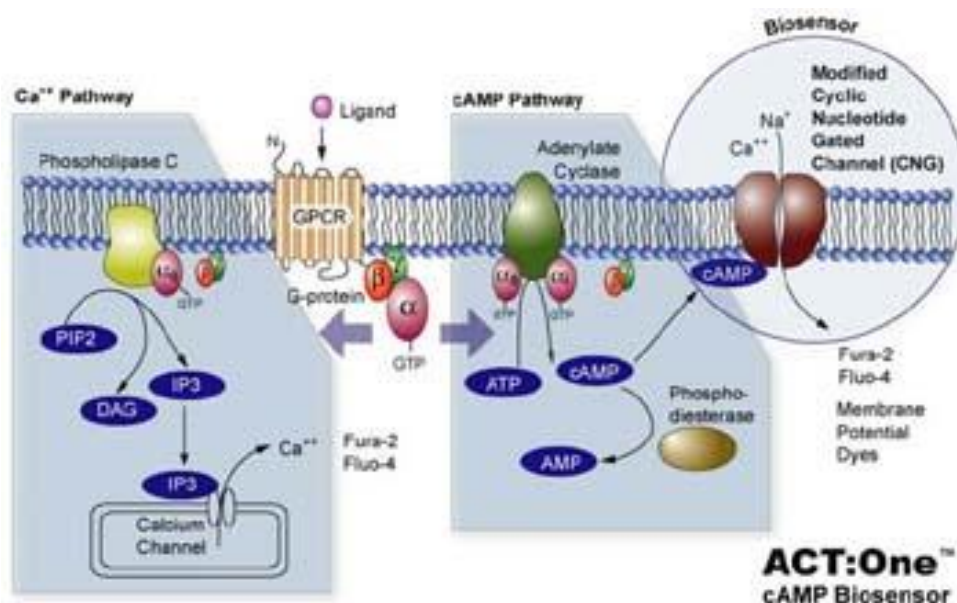
CATALOG NUMBER: CL-01-MC1R

Introduction

MC1R is a G protein-coupled receptor that binds to a class of pituitary peptide hormones known as the melanocortins, which include adrenocorticotrophic hormone (ACTH) and the different forms of melanocyte-stimulating hormone (MSH). MC1R is one of the key proteins involved in regulating mammalian skin and hair color. It is located on the plasma membrane of specialized cells known as melanocytes, which produce the pigment melanin through a process referred to as melanogenesis. It works by controlling the type of melanin being produced, and its activation causes the melanocyte to switch from generating the yellow or red pheomelanin by default to the brown or black eumelanin in replacement.

Description

Human MC1R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human MC1R. HEK-293 CNG cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, Cat# CA-C155) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye ([Cat# CA-M165](#)). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG cells (originally developed by BD Biosciences by introducing CNG in HEK-293 cells) (Cat# CL-03-PC20)

Gene/Enzyme Introduced

MC1R (Genbank Accession No. AF153431)

Applications

- cAMP dependent human MC1R cell based assay
- cell based high-throughput screening of human MC1R inhibitors

Functional Test

- this cell line has been tested positive for MC1R specific response
- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

- Stable cells: 1 mL (1 x 10⁶ cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 µg/ml G418, 1 µg/ml Puromycin
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Data Analysis

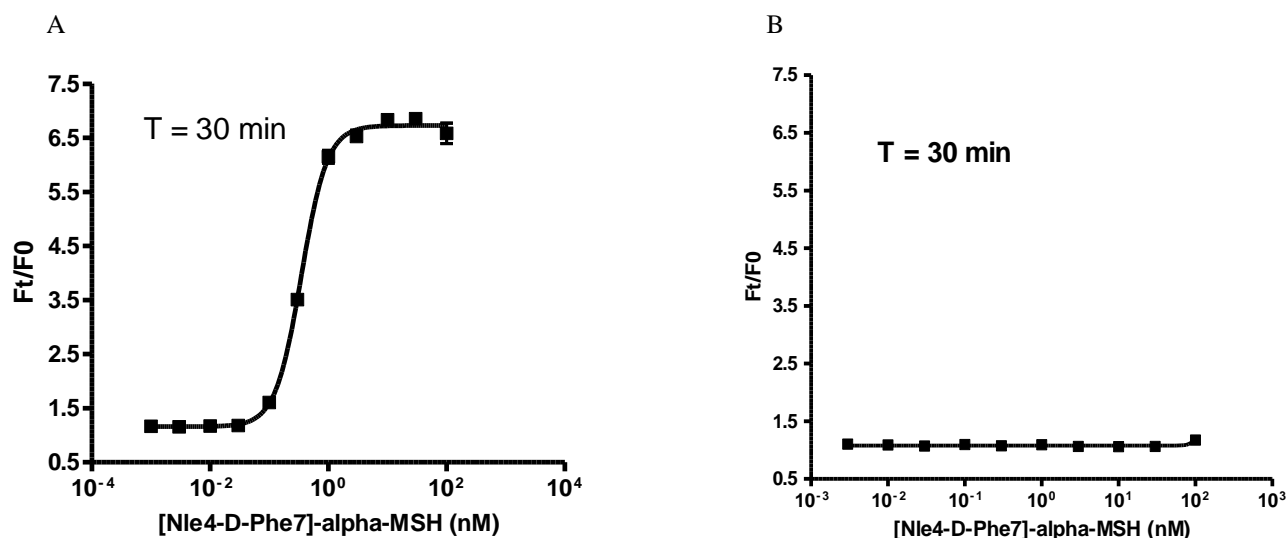


Figure 1. Response of ACTOne™ MC1R cell line & parental cell line to [Nle4, D-Phe7]α-MSH

ACTOne™ MC1R cells and parental cells (Cat# CL-03-PC20) 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 30 min after the addition of [Nle4, D-Phe7]α-MSH. Ratios of the two readings (Ft/F0) are plotted in the figure.

- Dose response curve of [Nle4, D-Phe7]α-MSH in ACTOne™ MC1R cell line. EC50 = 0.35 nM in the presence of PDE inhibitor Ro 20-1724.**
- Parental cells do not respond to [Nle4, D-Phe7]α-MSH.**

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