Melanocortin 5 Receptor (MC5R) ACTOne™ Stable Cell Line

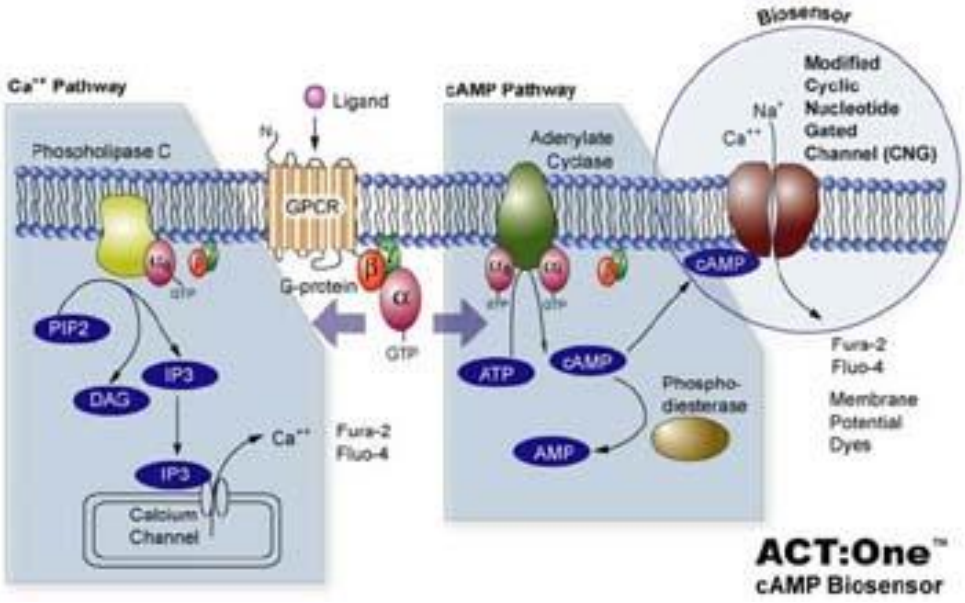
CATALOG NUMBER: CL-01-MC5R

Introduction

MC5R is a member of the seven-pass transmembrane G protein-coupled melanocortin receptor protein family that stimulate cAMP signal transduction. The encoded protein is a receptor for melanocyte-stimulating hormone and adrenocorticotropic hormone and is suggested to play a role in sebum generation.

Description

Human MC5R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human MC5R. 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

MC5R (Genbank Accession No. NP_005904.1)

Applications

- cAMP dependent human MC5R cell based assay
- cell based high-throughput screening of human MC5R inhibitors

Functional Test

- this cell line has been tested positive for MC5R 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 10^6 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

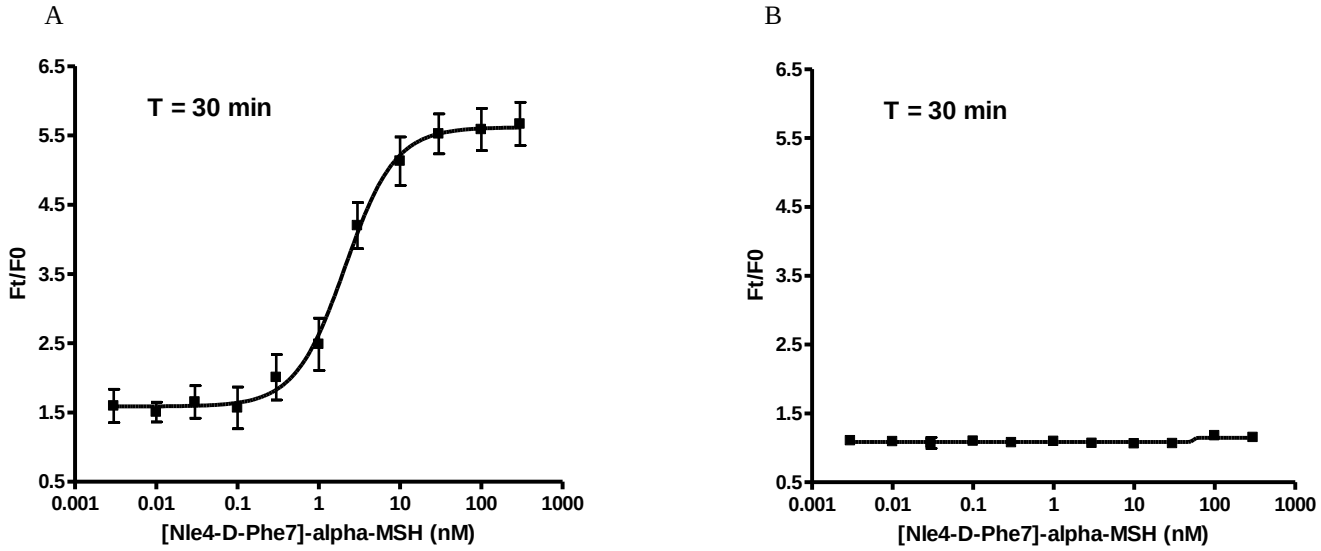


Figure 1. Response of ACT*One*™ MC5R cell line & parental cell line to [Nle4, D-Phe7]α-MSH

ACTOne™ MC5R 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 (F/F0) are plotted in the figure.

A. **Dose response curve of [Nle4, D-Phe7]α-MSH in ACT*One*™ MC5R cell line. EC50 = 2.1 nM in the presence of PDE inhibitor Ro 20-1724.**
B. **Parental cells do not respond to [Nle4, D-Phe7]α-MSH.**

Notice to Purchaser

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