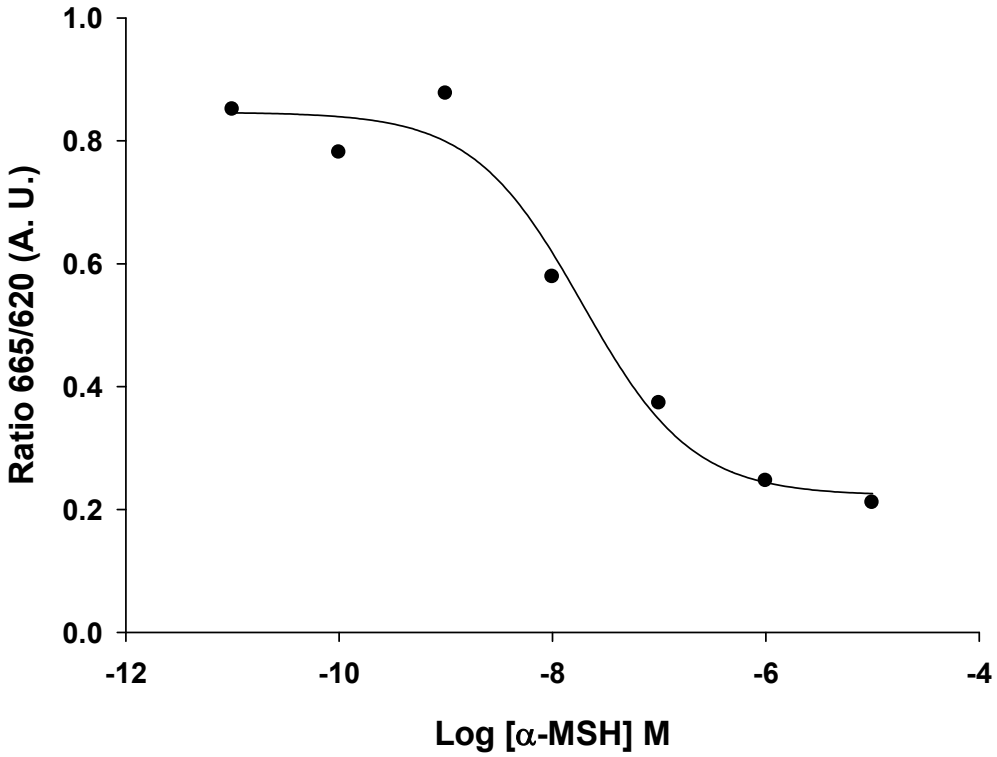
REF: P30410

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- MELANOCORTIN 4 RECEPTOR CELL LINE -



Product name: MC4R /HEK293 cell line

Ec_{50} α-MSH: $1.90x10^{-8}$ M

Z´: 0.81+/- 0.02

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- MELANOCORTIN 4 RECEPTOR CELL LINE -

Product Name:	MC4R/HEK293
Official Full Name:	Melanocortin 4 receptor
DNA Accesion Number:	GenBank: AY236539.1
Host Cell:	HEK293
Format:	2 cryopreserved vials
Resistance:	Puromycin
References:	
P30410	2 vials of 3 x 10^6 proliferative cells
P30410-DA	1 vial of 2.5 x 10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker MC4R contains HEK293 cells stably expressing human Melanocortin 4 Receptor with no tag.

Innoprot HiTSeeker MC4R cell line has been designed to assay compounds or analyze their capability to modulate Melanocortin 4 Receptor. When the agonist binds to MC4R, a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (cAMP).

This cell line has been validated measuring cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring MC4R activation process in High Throughput Screening.

About MC4R

MC4R is a membrane-bound receptor and part of the Melanocortin Receptors family, that are members of the rhodopsin family receptors. MC4R binds adrenocorticotropic and α-melanocyte stimulating hormone (α-MSH) and it is involved in a wide range of physiological functions as energy balance, metabolism regulation, feeding and sexual behaviour and erectile function. Mutations in its gene are associated with autosomal dominant obesity. It is mainly expressed in brain, placenta and intestinal tissue.

When α-MSH binds to its receptor, a G-protein signal cascade activates adenylyl cyclase and intercellular levels of cAMP rise.

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Assay Characterization

Our expression plasmid contains the coding sequence of human MC4R protein. Our plasmid was transfected in HEK293 cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

Fig.1. GAPDH housekeeping gene and MC4R gene RT-PCR.

Validation of MC4R cell line

cAMP production assay (Ec50= 1.90x10^{-8} M)

cAMP production was assessed using the cAMP dynamic 2 kit (Cisbio). This kit contains labelled cAMP (620 nm) and an anti-cAMP antibody (665nm). Between these molecules occurs a fluorescence transfer (FRET). Native cAMP produced by cells (due to the binding of an agonist to its specific receptor) competes with the labelled cAMP producing a decrease of FRET detected by HTRF technology.

The specific signal is inversely proportional to the concentration of native cAMP produced by the binding of the agonist to its receptor (Fig.2).

Fluorescence detection was recorded in a Multi-Mode Microplate Reader Synergy 2 from Biotek.

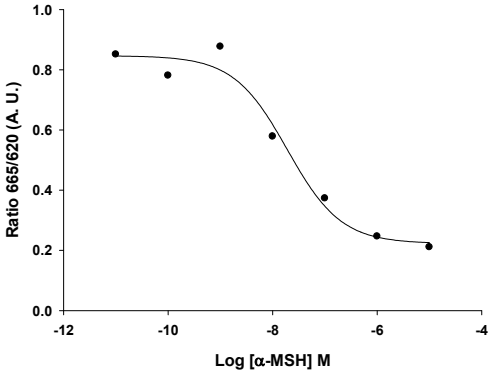

Fig.2. α-MSH dose response in cAMP assay. Cells were treated with **α-MSH** concentrations ranging from 0 to 10 µM, n=3. The EC50 for **α-MSH** was 1.90x10^{-8} M. The cAMP assay was validated with a Z´= 0.81+/- 0.02 for High Content Screening.

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