innoprot
cell based solutions

NOMAD CELL LINES

P70554

Nomad Biosensors™ comprise a family of genetically encoded fluorescent sensors designed to monitor the signaling of G protein-coupled receptors (GPCRs) in cell-based assays.

Nomad Biosensors™ are engineered to measure the intracellular dynamics of second messengers such as calcium (Ca^{2+} Nomad), cAMP (cAMP Nomad), or diacylglycerol (DAG Nomad) upon GPCR activation. Additionally, β-arrestin signaling can also be studied using these biosensors. Nomad Biosensors™ can be combined in the same cell line for multiplex assays.

Prior to GPCR activation, the biosensors are localized in the plasma membrane. Upon ligand binding, the sensors undergo a conformational change that leads to an increase in fluorescence intensity and their relocalization within the vesicular trafficking pathways of the cells.

cAMPNOMAD MC4R

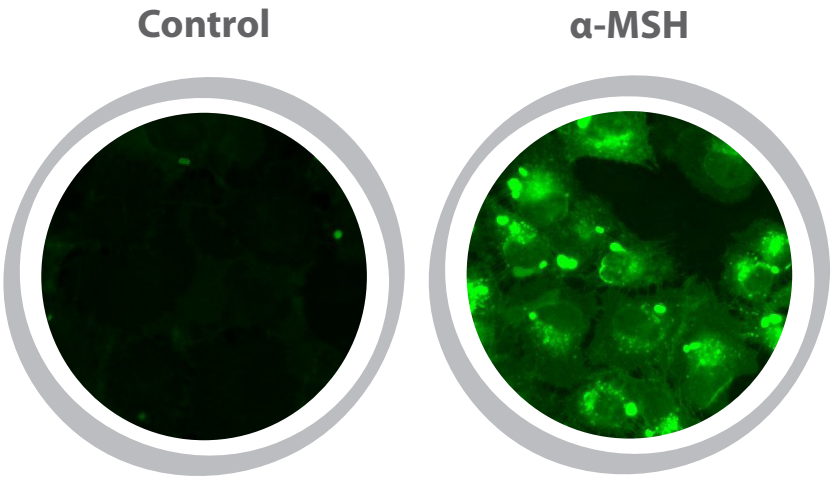
cAMP Assay

Product Name: cAMPNomad-MC4R Cell Line
Reference: P70554
Gene Name: Melanocortin 4 Receptor (MC4R)
cDNA Accession Number: AY236539
Host Cell Line: U2OS
Selection Markers: Geneticin (G418) + Hygromycin
Cell Quantity: > $3x10^6$ cells/vial
Storage Conditions: Liquid Nitrogen

About cAMPNomad-MC4R

Nomad cell lines are a reliable system for studying G protein-coupled receptor (GPCR) signaling in living cells.

Optimized for the integration into High Content Screening (HCS) and High Throughput Screening (HTS) workflows, cAMPNomad-MC4R Cell Line stably express green cAMPNomad Biosensor along with the Melanocortin 4 Receptor (MC4R).



NOMAD CELL LINES

cAMP Agonism & Antagonism Assays

The cAMPNomad-MC4R Cell Line was plated in a 96-well plate and incubated for a minimum of 4 hours and up to 24 hours at 37°C with 5% CO_2 to allow the cells to attach to the plate surface.

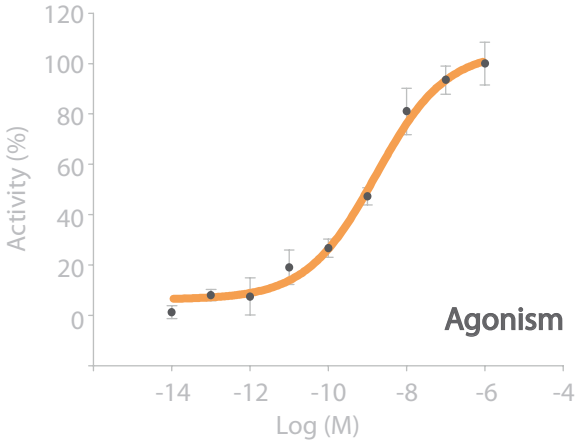
Agonism Assay: Cells were incubated with α-MSH diluted in a serum-reduced medium for 20–24 hours.

Antagonism Assay: Cells were incubated with HS024 diluted in 1 µM α-MSH serum-reduced medium for 20–24 hours.

The increase (Agonism Assay) or decrease (Antagonism Assay) in the fluorescence intensity of the green cAMPNomad biosensor (% Activity) was detected and analyzed using a microplate reader.

EC_{50} α-MSH: 1.70×10^{-9} M

Z´: 0.67+/- 0.01



IC_{50} HS024 : 3.64×10^{-5} M

Z´: 0.64+/- 0.01

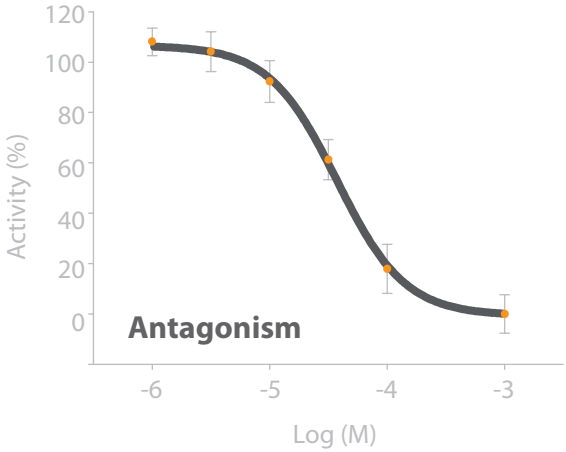


Figure 1. Dose-response curves for MC4R ligands.
Top: *concentration response curve for α-MSH in the agonism assay.*
Bottom: *concentration response curve for HS024 for the antagonism assay.*
The % Activity corresponds to the fluorescence intensity emitted by the green cAMPNomad biosensor normalized against the controls.

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