



# ICAMPNOMAD M4

## P70545-01R

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

Nomad Biosensors™ are engineered to measure the intracellular dynamics of second messengers such as calcium (Ca²+ 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.

## icAMP Assay

**Product Name:** icAMPNomad-M4 Receptor Cell Line

Reference: P70545-01R

**Gene Name:** Cholinergic receptor muscarinic 4 (M4)

**cDNA Accession Number:** X15265.1

Host Cell Line: HEK 293

**Selection Markers:** Geneticin (G418) + Puromycin

**Cell Quantity:** > 3x10<sup>6</sup> cells/vial

**Storage Conditions:** Liquid Nitrogen

#### About icampNomad-M4

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, icampNomad-M4 Receptor Cell Line stably express red icampNomad Biosensor along with the Cholinergic receptor muscarinic 4 (M4).

Control Oxotremorine





### icAMP Agonism & Antagonism Assays

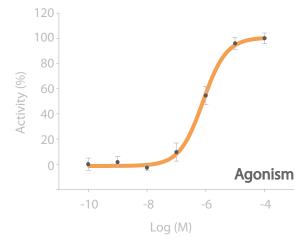
The icampNomad-M4 Receptor Cell Line was plated in a 96-well plate and incubated for a minimum of 4 hours and up to 24 hours at 37°C with 5% CO<sub>2</sub> to allow the cells to attach to the plate surface.

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

**Antagonism Assay:** Cells were incubated with atropine diluted in 10  $\mu$ M oxotremorine serum-reduced medium for 20–24 hours.

The increase (Agonism Assay) or decrease (Antagonism Assay) in the fluorescence intensity of the red <code>icampNomad</code> biosensor (% Activity) was detected and analyzed using a microplate reader.

EC<sub>50</sub> Oxotremorine: 8.19x10<sup>-7</sup> M Z': 0.63+/- 0.01



IC₅o Atropine: 6.79x10<sup>-7</sup> M Z': 0.63+/- 0.01

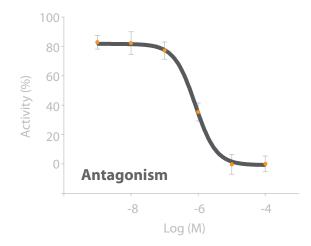


Figure 1. Dose-response curves for M4 ligands.

**Top:** concentration response curve for oxotremorine in the agonism assay.

**Bottom:** concentration response curve for atropine for the antagonism assay.

The % Activity corresponds to the fluorescence intensity emitted by the red icampNomad biosensor normalized against the controls.



