innoprot
cell based solutions

NOMAD CELL LINES

# P70545-02R

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.

# icAMPNOMAD M4

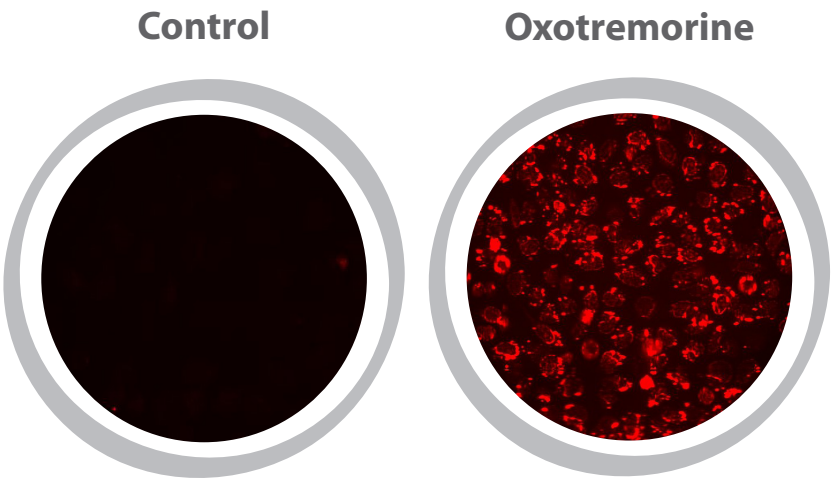
## icAMP Assay

**Product Name:** icAMPNomad-M4 Receptor Cell Line
**Reference:** P70545-02R
**Gene Name:** Cholinergic receptor muscarinic 4 (M4)
**cDNA Accession Number:** X15265.1
**Host Cell Line:** U2OS
**Selection Markers:** Geneticin (G418 ) + Puromycin
**Cell Quantity:** > $3x10^6$ 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).



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## 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% $CO_2$ to allow the cells to attach to the plate surface.

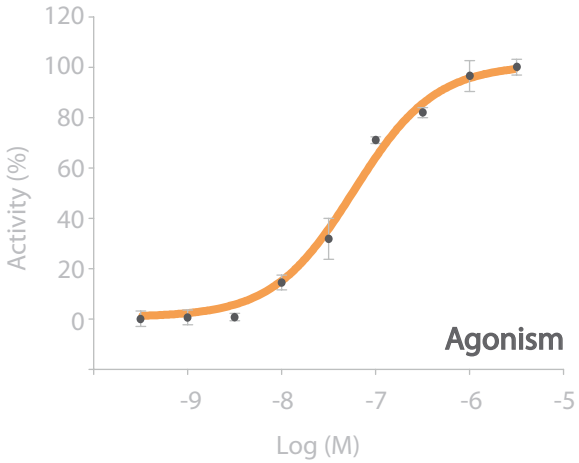
**Agonism Assay**: Cells were incubated with Oxotremorine diluted in a serum-reduced medium for 20–24 hours.

**Antagonism Assay**: Cells were incubated with atropine diluted in 300 nM oxotremorine serum-reduced medium for 20–24 hours.

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

$EC_{50}$ Oxotremorine: $4.27x10^{-8}$ M

Z´: 0.87+/- 0.01




$IC_{50}$ Atropine: $2.14x10^{-9}$ M

Z´: 0.96+/- 0.01

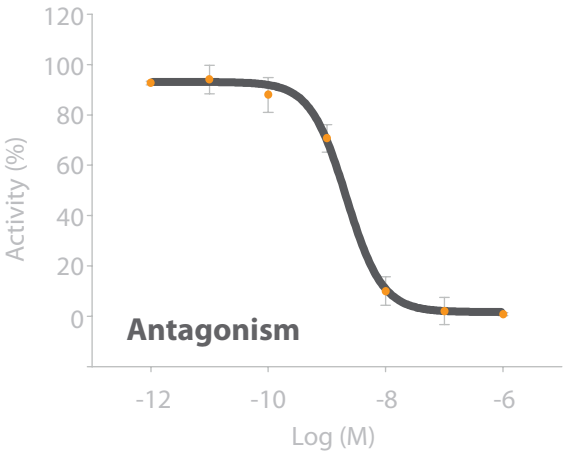



***Figure 1. Dose-response curves for M4 ligands.***
***Top:*** *concentration response curve for oxotremorine in the agonism assay.*
***Bottom:*** *concentration response curve for atropine for the antagonism assay.*
*The % Activity corresponds to the fluorescence intensity emitted by the red icAMPNomad biosensor normalized against the controls.*

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