

Ref. P70701

Nomad Biosensors are genetically encoded fluorescent biosensors that measure changes in second messengers (Ca^{2+} , cAMP or DAG) and β -arrestin signaling pathways.

Before the stimulation mediated by the ligand of interest, the fluorescent biosensors are localized in the cellular membrane.

Upon ligand binding, the Nomad biosensors modify their structural folding. This conformational change promotes a cellular relocation of the biosensors in the vesicular trafficking and a subsequent increase in the fluorescence intensity emitted.

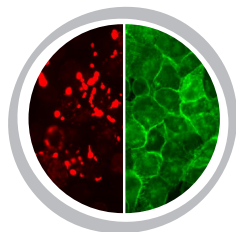
In a multiplex cell line coexpressing Nomad biosensors (β -arrestin - Ca^{2+}) and a GPCR, the activity of the receptor can be easily quantified on living cells by image analysis in a HCS equipment or by fluorescence emission in a microplate reader for HTS assays.



Innoprot

MPX NOMAD EP₁/PTGER1 (PROSTAGLANDIN E RECEPTOR 1)

Calcium and β -Arrestin ASSAY



Product Name: MPXNomad-EP₁ cell line

Reference: P70701

Recp. Official Full Name: Prostaglandin E receptor 1

DNA Accession Number: AY275470

Host Cell: U2OS

Resistance: G418 + Puromycin + Hygromycin

Quantity: > 3 × 10⁶ cells / vial

Storage: Liquid Nitrogen



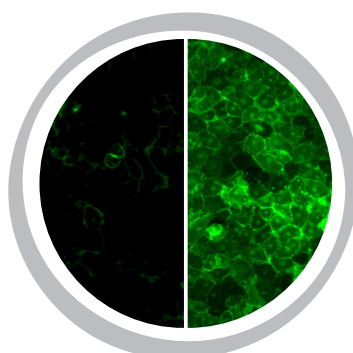
About MPXNomad-EP₁ (U2OS cell line)

Each vial of MPXNomad-EP₁ cell line contains U2OS cells that express, in addition to the red β -Arrestin and the green Ca^{2+} Nomad biosensors, the Prostaglandin E receptor 1 (untagged).

This cell line has been designed to assay compounds or analyze their capability to modulate the activity of the Prostaglandin E receptor 1. When a ligand binds to the EP₁ receptor a G protein is activated, which in turn, triggers a cellular response mediated by Ca^{2+} and a subsequent internalization mediated by β -Arrestin. Prostaglandin E receptor 1 signaling has been analyzed measuring the fluorescence intensity changes emitted by the Nomad biosensors upon ligand binding.

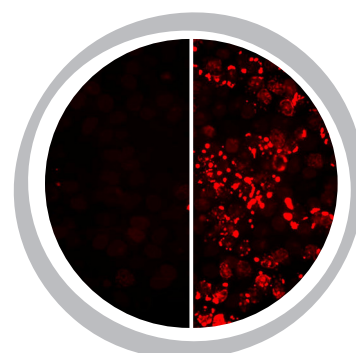
This highly reproducible assay has been validated using Iloprost or PGE₂ as agonists and AH6809 as antagonist of the EP₁ receptor in High Throughput Analyses (HTAs).

Ca^{2+}



Control | PGE₂

β -Arrestin



Control | PGE₂



β -Arrestin and Calcium Agonism Assays

β -Arrestin- Ca^{2+} MPXNomad U2OS cells, stably expressing Prostaglandin E receptor 1 (EP_1), were stimulated with increasing dilutions of Iloprost or PGE_2 during 24h ($n=5$) (Fig. 1). The data were normalized as percentages of activity against the positive control after subtracting the value of the vehicle control.



Fluorescence intensity analysis

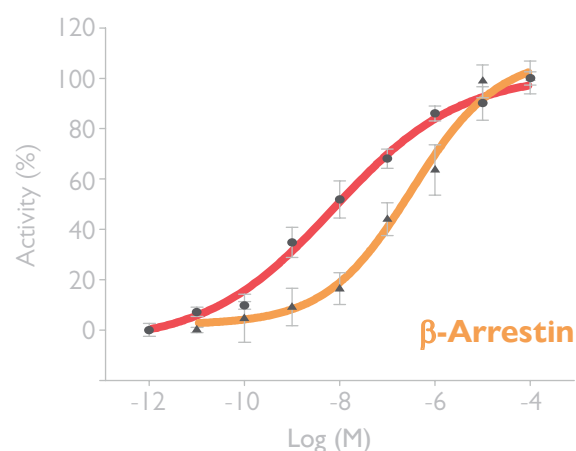
The increase in the emitted fluorescence was detected and analyzed using a fluorescence microplate reader. The EC_{50} value and Z' factor were calculated for PGE_2 (red and green lines) and for Iloprost (orange and grey lines).

EC_{50} Iloprost: 3.29×10^{-7} M

Z' : 0.76 ± 0.01

EC_{50} PGE_2 : 7.33×10^{-9} M

Z' : 0.84 ± 0.01



EC_{50} Iloprost: 1.52×10^{-7} M

Z' : 0.70 ± 0.01

EC_{50} PGE_2 : 2.57×10^{-9} M

Z' : 0.62 ± 0.01

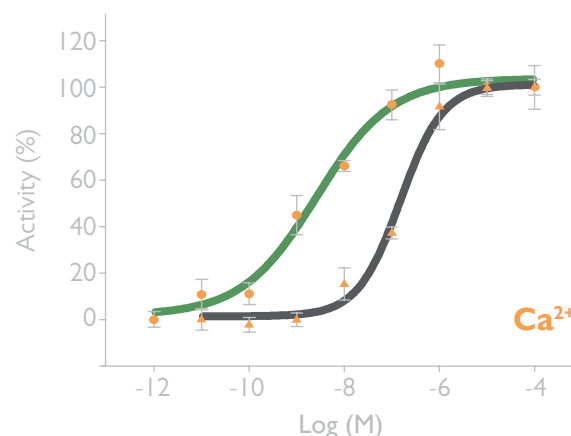


Figure 1. Dose-response curves for PTGER1 agonists. Top: concentration response curves for PGE_2 (red line) and Iloprost (orange line) for the red arrestin biosensor. Bottom: Concentration response curves for PGE_2 (green line) and Iloprost (grey line) for the green Ca^{2+} biosensor. The % Activity corresponds to the fluorescence intensity emitted by the Nomad biosensors normalized against the controls.



β -Arrestin and Calcium Antagonism Assays

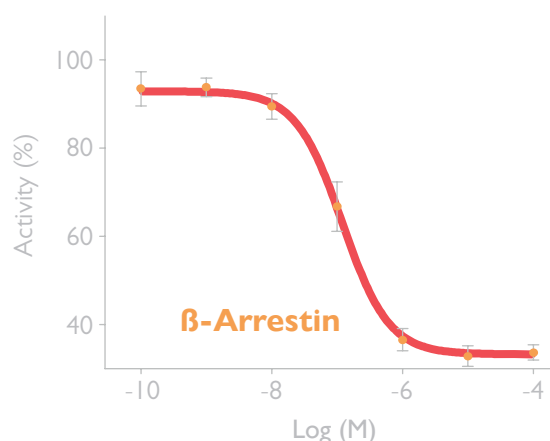
β -Arrestin- Ca^{2+} MPXNomad U2OS cells, stably expressing Prostaglandin E receptor 1 (EP_1), were co-treated with increasing dilutions of AH-6809 and 100 nM PGE_2 during 24h (n=5) (Fig. 2). The data were normalized as percentages of activity against the positive control after subtracting the value of the vehicle control.



Fluorescence intensity analysis

The decrease in the emitted fluorescence was detected and analyzed using a fluorescence microplate reader. The IC_{50} value and Z' factor were calculated for AH-6809 (red and green lines).

IC_{50} AH-6809: 1.11×10^{-7} M
 Z' : 0.72 ± 0.01



IC_{50} AH-6809: 1.02×10^{-7} M
 Z' : 0.84 ± 0.01

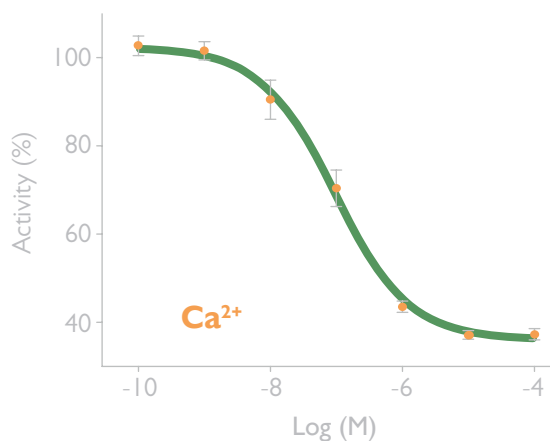


Figure 2. Dose-response curves for PTGER1 antagonist. Top: concentration response curve for AH6809 (red line) for the red arrestin biosensor. Bottom: Concentration response curve for AH6809 (green line) for the green Ca^{2+} biosensor. The % Activity corresponds to the fluorescence intensity emitted by the Nomad biosensors normalized against the controls.