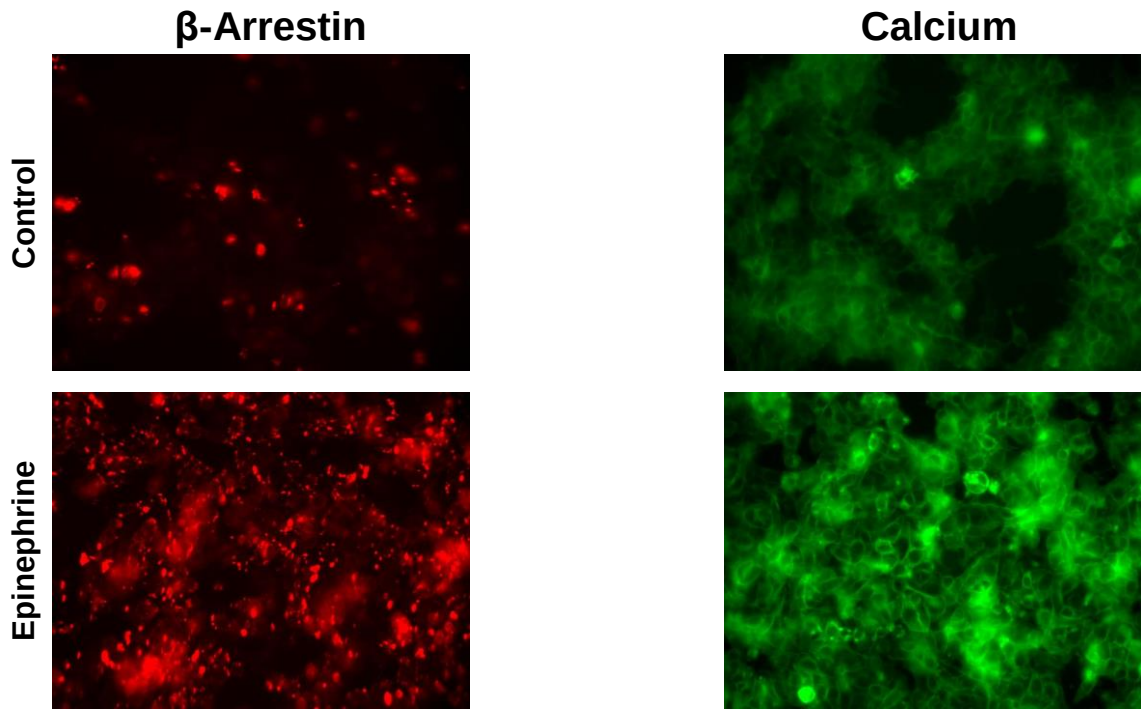
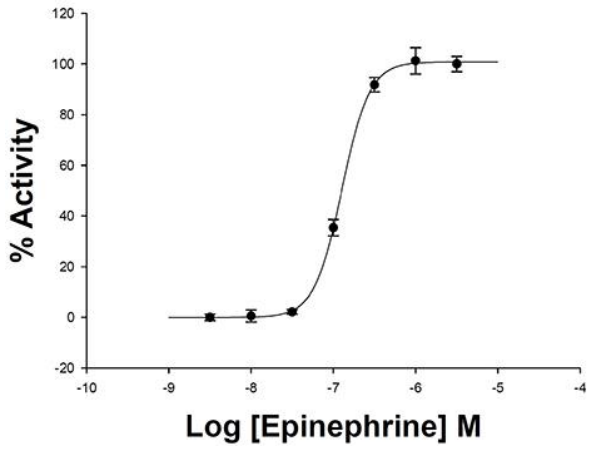
REF: P70741

NOMAD CELL LINES – Calcium and ß-Arrestin

MPXNOMAD ADRENOCEPTOR ALPHA 1B (ADRA1B)

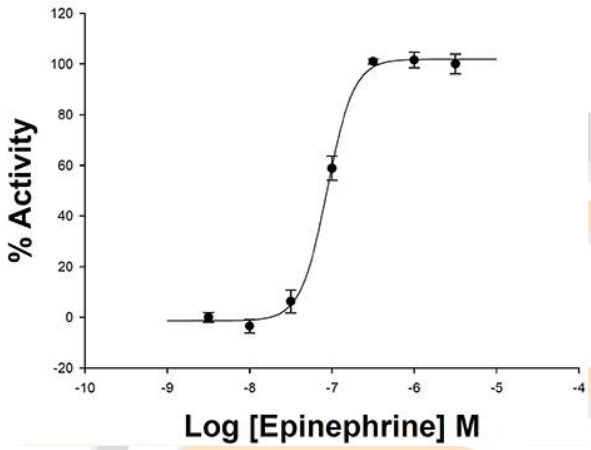
MPXNomad-ADRA1B (HEK293 cell line)





EC_{50} ß-Arrestin1 assay: $1.27x10^{-7}$ M

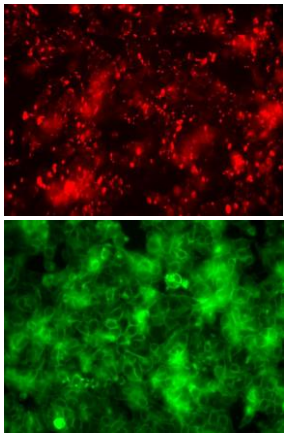
$Z'_{ß\text{-}Arrestin1}$: 0.87



EC_{50} Calcium assay: $8.76x10^{-8}$ M

$Z'_{Calcium}$: 0.83

VAT No. ESB95481909
INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edificio 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com



Product Name: $_{MPX}$Nomad-ADRA1B cell line

Reference: P70741

Receptor Official Full Name: Adrenoceptor alpha 1B

DNA Accession Number: NM_000679

Host Cell: HEK293

Resistance: Puromycin + G618

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of $_{MPX}$Nomad-ADRA1B contains HEK293 cells stably expressing red $_{ß\text{-}Arrestin}$Nomad and green $_{Ca2+}$Nomad biosensor and Adrenoceptor alpha 1B receptor (no tag).

Innoprot´s $_{MPX}$Nomad-ADRA1B cell line has been designed to assay compounds or analyze their capability to modulate Adrenoceptor alpha 1B receptor. When an agonist binds to ADRA1B a G protein is activated which, in turn, triggers a cellular response mediated by calcium and a subsequent internalization mediated by ß-Arrestin.

This cell line has been validated measuring calcium signalling and ß-Arrestin mobilization analyzing Nomad biosensors distribution within the cell.

This highly reproducible assay has been validated using human Neurotensin as agonist in a High Content Analysis (HCA) and a High Throughput Screening (HTS).

About Nomad Biosensor Family

Nomad Biosensor family is based in a fluorescent polypeptide that measures fluctuations in the calcium and Arrestin signalling pathways, changing its localization and fluorescent intensity emission within the cell.

Before the stimulation mediated by the agonist of interest, the fluorescent biosensors are located in the cellular membrane. An increase in the second messenger concentration leads to a change in the structural folding of the Nomad Biosensors that promotes their cellular relocation in the vesicular trafficking of the cells and an increase in the fluorescence.

In a cell line co-expressing $_{MPX}$Nomad Biosensor (calcium + β-arrestin) and a GPCR, the activity can be easily quantified on living cells by image analysis or fluorescence emission in a microplate reader.

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innoprot@innoprot.com | www.innoprot.com

ß-Arrestin & cAMP ASSAY

ß-arrestin-Ca^{2+} $_{MPX}$Nomad HEK293 cells, stably expressing Adrenoceptor alpha 1A receptor (ADRA1B), were stimulated with 10 dilution series ranging from 30 µM to 5 nM of Epinephrin during 24h (n=8). % Activity was calculated relative to positive.

Fluorescence intensity analysis

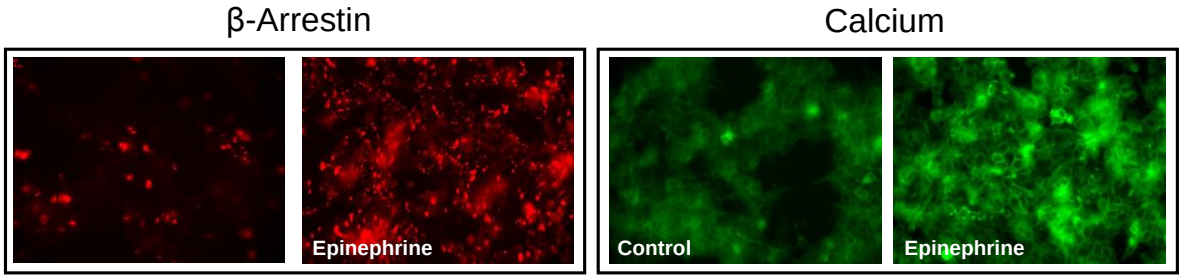

Fig 1. ß-arrestin-Ca^{2+} $_{MPX}$Nomad biosensor stimulated with 30 µM of Epinephrine. *Left (red)*: ß-arrestin biosensor; *Right* (*green*): Ca2+ biosensor.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for Epinephrine after a treatment of 24 h was $1.27x10^{-7}$ M for the ß-arrestin assay (validated with a Z´= 0.87) and $8.76x10^{-8}$ M for the calcium assay (Z´=0.83).

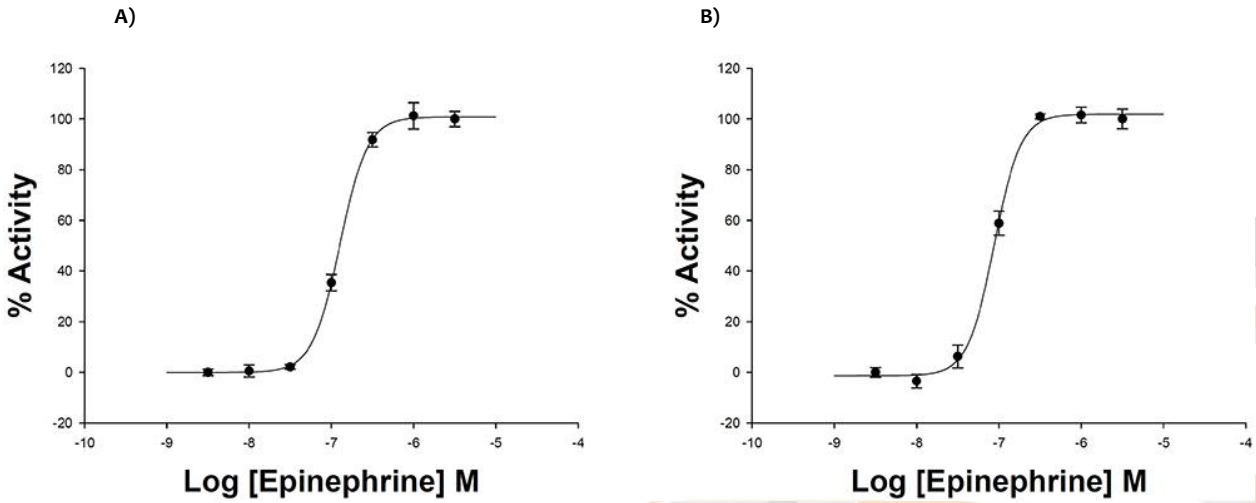

Fig 2. Concentration-response curve for Epinephrine in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-ADRA1B cell line analyzed using the "Synergy 2" microplate reader (Biotek). **A)** Concentration response curve for Epinephrine for red arrestin biosensor. **B)** Concentration response curve for Epinephrine for green calcium biosensor.

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