

NOMAD CELL LINES – Calcium and β -Arrestin

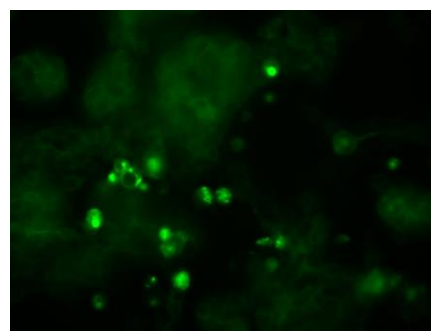
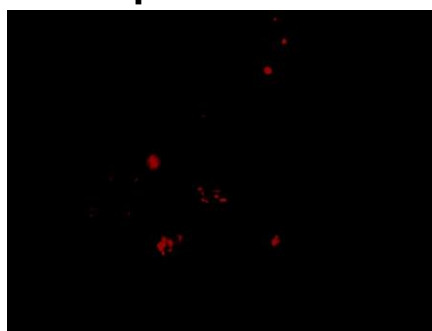
MPX NOMAD ADRENOCEPTOR ALPHA 1A (ADRA1A)

MPXNomad-ADRA1A (HEK293 cell line)

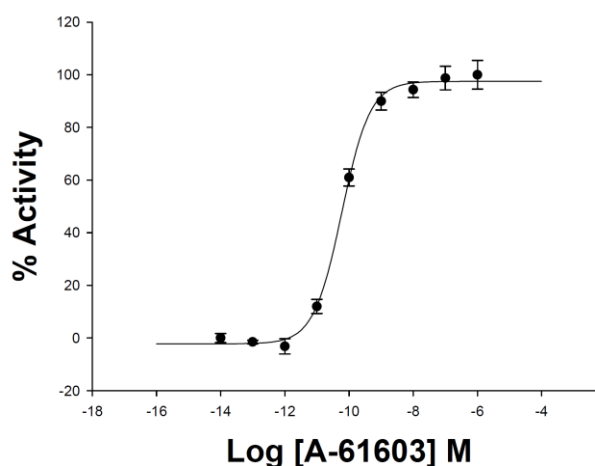
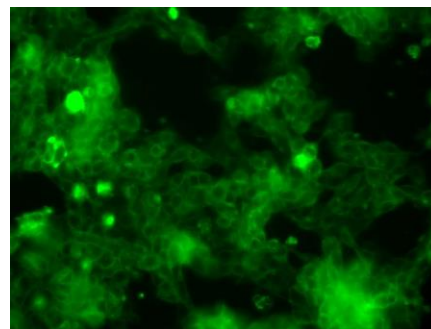
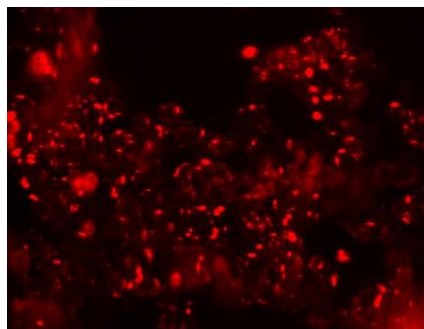
β -Arrestin

Calcium

Control

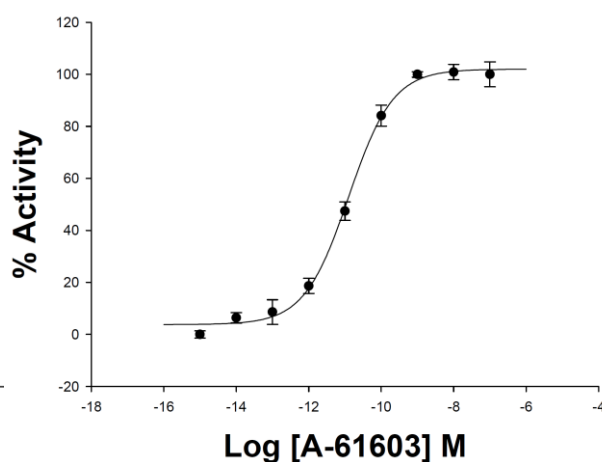


A-61603



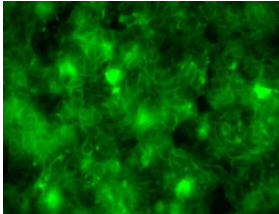
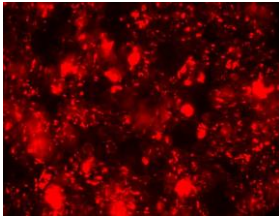
EC_{50} β -Arrestin1 assay: 5.97×10^{-11} M

Z' β -Arrestin: 0.79



EC_{50} Calcium assay: 1.26×10^{-11} M

Z' Calcium: 0.82



Product Name: MPX Nomad-ADRA1A cell line

Reference: P70740

Receptor Official Full Name: Adrenoceptor alpha 1A

DNA Accession Number: AY_389505

Host Cell: HEK293

Resistance: Puromycin + G418

Quantity: $> 3 \times 10^6$ cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of MPX Nomad-ADRA1A contains HEK293 cells stably expressing red β -ArrestinNomad and green Ca^{2+} -Nomad biosensor and Adrenoceptor alpha 1A receptor (no tag).

Innoprot's MPX Nomad-ADRA1A cell line has been designed to assay compounds or analyze their capability to modulate Adrenoceptor alpha 1A receptor. When an agonist binds to ADRA1A a G protein is activated which, in turn, triggers a cellular response mediated by calcium and a subsequent internalization mediated by β -Arrestin.

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

This highly reproducible assay has been validated using A-61603 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, fluorescent Nomad biosensors are located in the cellular membrane showing a low fluorescence intensity. 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.

β -Arrestin & cAMP ASSAY

β -arrestin- Ca^{2+} MPXNomad HEK293 cells, stably expressing Adrenoceptor alpha 1A receptor (ADRA1A), were stimulated with 10 dilution series ranging from 0 to 10 μM of A-61603 during 24h (n=8). % Activity was calculated relative to positive.

Fluorescence intensity analysis

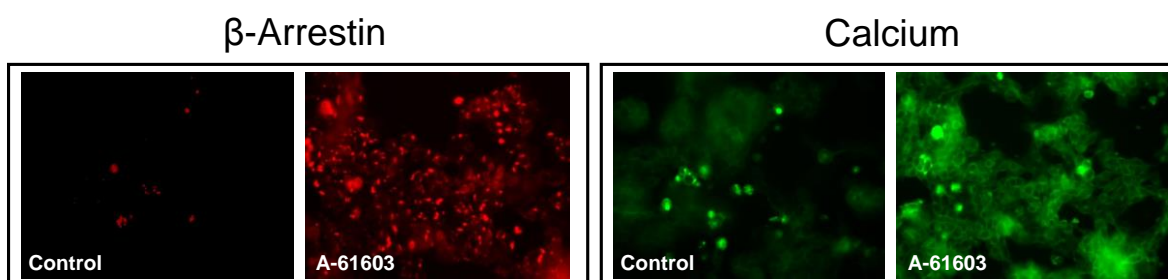


Fig 1. β -arrestin- Ca^{2+} MPXNomad biosensor stimulated with 10 μM of A-61603. Left (red): β -arrestin biosensor; Right (green): Ca^{2+} biosensor.

The increase in the fluorescence was detected and analyzed using “Synergy 2” microplate reader from Biotek. The EC_{50} for A-61603 after a treatment of 24 h was 5.97×10^{-11} M for the β -arrestin assay (validated with a $Z' = 0.79$) and 1.26×10^{-11} M for the calcium assay ($Z' = 0.82$).

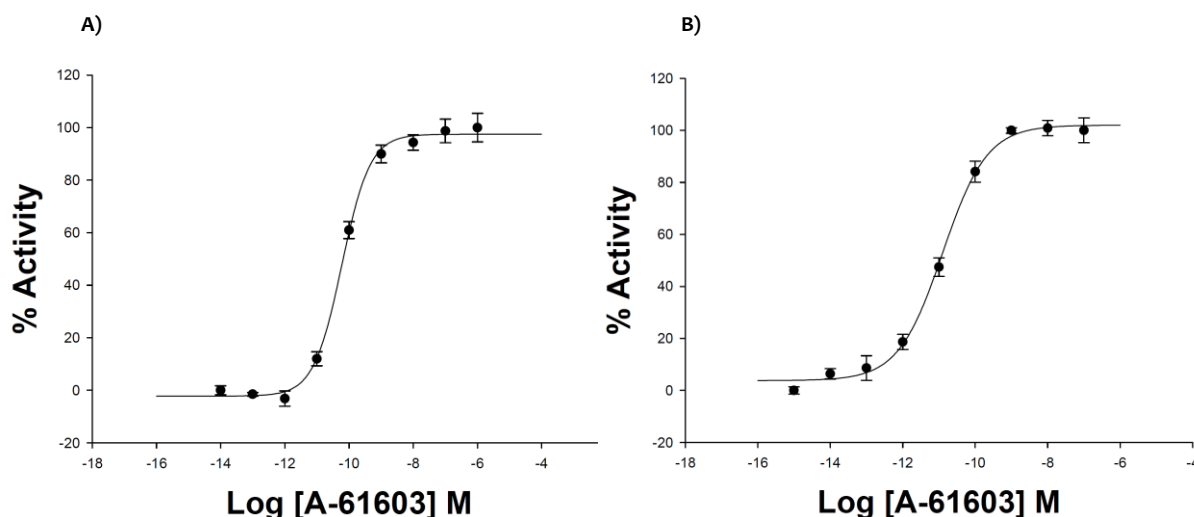


Fig 2. Concentration-response curve for A-61603 in β -arrestin- Ca^{2+} MPXNomad -ADRA1A cell line analyzed using the “Synergy 2” microplate reader (Biotek). **A)** Concentration response curve for A-61603 for red arrestin biosensor. **B)** Concentration response curve for A-61603 for green calcium biosensor.