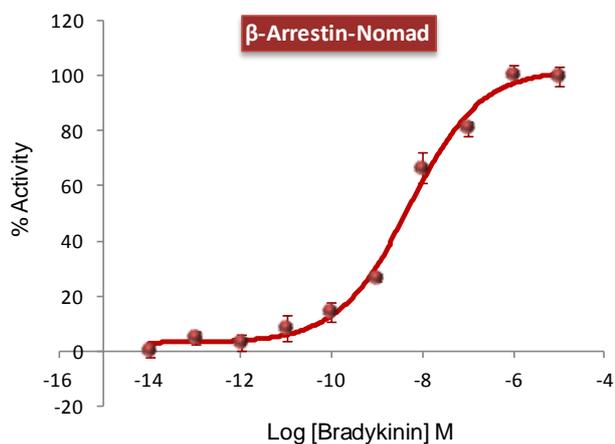
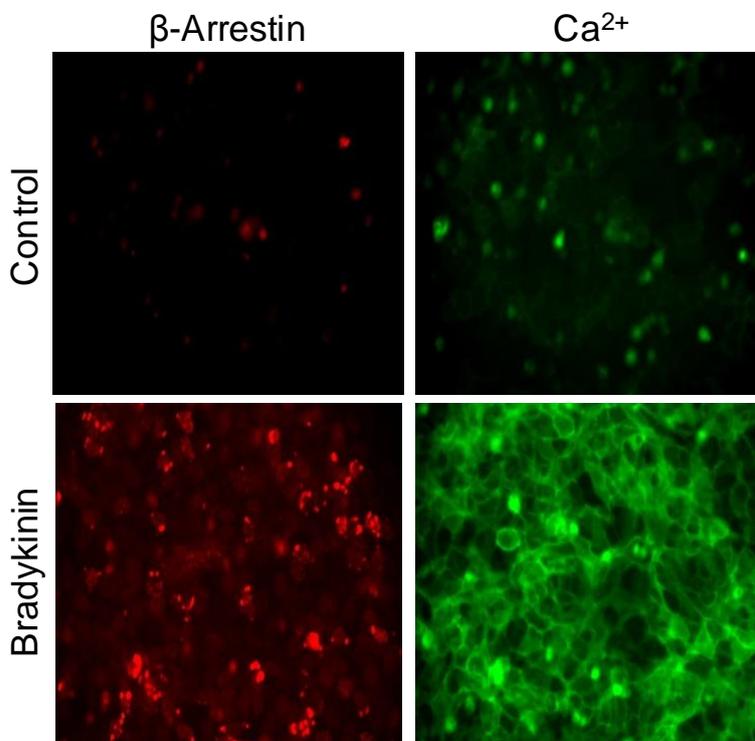
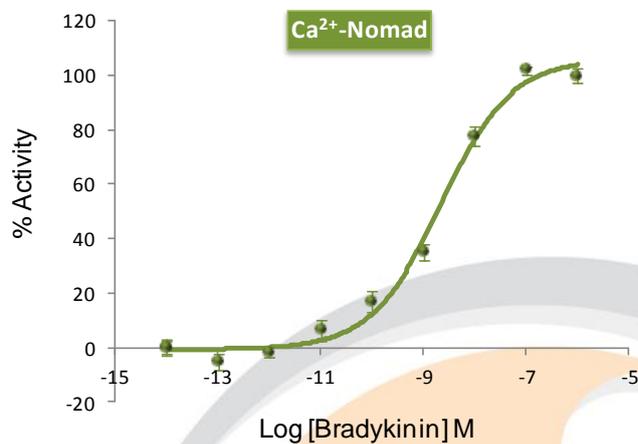


MULTIPLEX CELL LINES – β -Arrestin and Ca^{2+}
MPX NOMAD Bradykinin receptor B2 (BDKRB2)



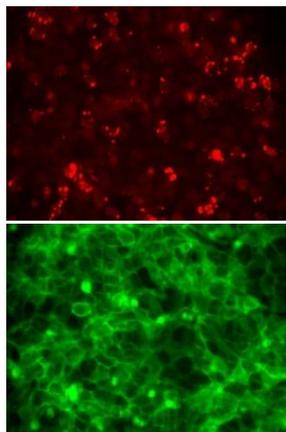
EC_{50} β -Arrestin assay: 5.21×10^{-9} M

Z' β -Arrestin: 0.83



EC_{50} Ca^{2+} assay: 2.18×10^{-9} M

Z' Ca^{2+} : 0.84



Product Name: MPX Nomad-BK2 cell line

Reference: P70709

Receptor Official Full Name: Bradykinin receptor B2

Host Cell: U2OS

Resistance: Puromycin + G418 + Hygromycin

DNA Accession Number: NM_000623

Quantity: > 3 x 10⁶ cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of MPX Nomad-BK2 contains U2OS cells stably expressing red β -Arrestin1Nomad and green Ca^{2+} Nomad biosensor and Bradykinin receptor B2 (with no tag).

Innoprot's MPX Nomad-BK2 cell line has been designed to assay compounds or analyze their capability to modulate Bradykinin receptor B2. When an agonist binds to BK2 a G protein is activated which, in turn, triggers a cellular response mediated by Ca^{2+} and a subsequent internalization mediated by β -Arrestin.

This cell line has been validated measuring Ca^{2+} signaling and β -Arrestin mobilization analyzing Nomad biosensors distribution within the cell.

This highly reproducible assay has been validated using bradykinin as agonist in both a High Content Analysis (HCA) and a High Throughput Analysis (HTA).

About Nomad Biosensor Family

Nomad Biosensors are genetically encoded fluorescent biosensors that measure fluctuations in second messengers (Ca^{2+} , cAMP or DAG) and β -arrestin signaling pathways. Upon activation, the biosensors change their 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 (β -arrestin - Ca^{2+}) and a GPCR, the activity can be easily quantified on living cells by image analysis or fluorescence emission in a microplate reader.

MULTIPLEX CELL LINES – β -Arrestin and Ca^{2+}

β -Arrestin- Ca^{2+} _{MPX}Nomad U2OS cells, stably expressing Bradykinin receptor B2 (BK2), were stimulated with increasing dilutions ranging from 0 to 10 μ M of bradykinin during 24h (n=8) (Fig 1). The data were normalized as percentages of activity compared with the positive control (bradykinin) after subtracting the value of the vehicle control.

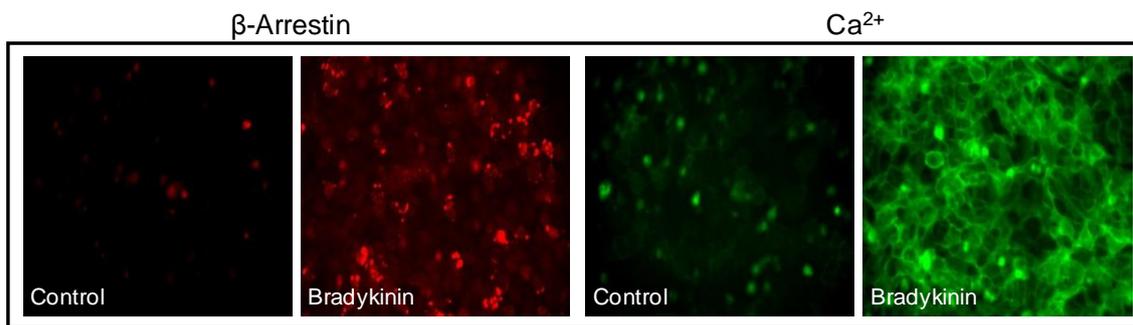


Fig 1. β -arrestin- Ca^{2+} _{MPX}Nomad biosensors stimulated with 10 μ M of bradykinin. *Left (red):* β -arrestin biosensor; *Right (green):* Ca^{2+} biosensor.

The increase in the fluorescence was detected and analyzed using the “Synergy 2” microplate reader from Biotek. The EC_{50} for bradykinin after a treatment of 24 h was 5.21×10^{-9} M for the β -arrestin assay (validated with a $Z' = 0.83$) and 2.18×10^{-9} M for the calcium assay ($Z' = 0.84$).

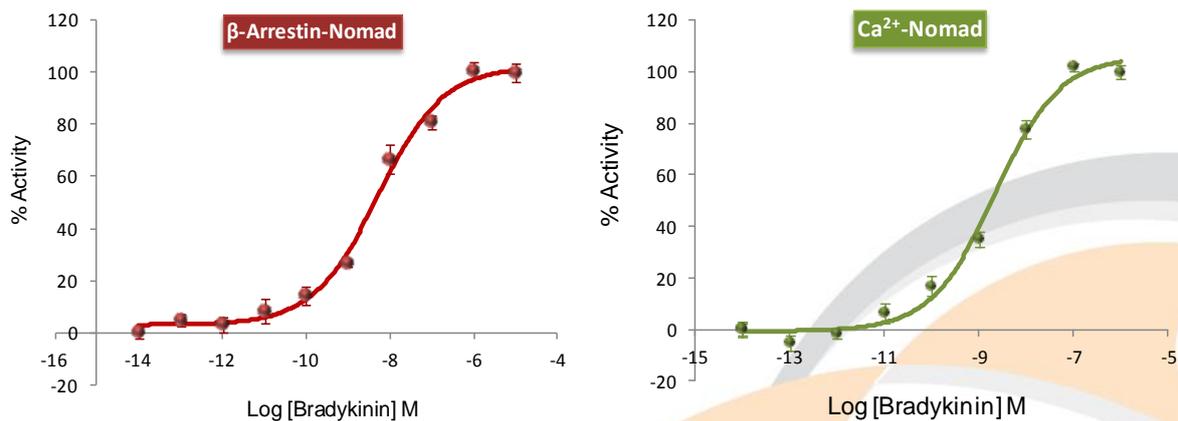


Fig 2. Concentration-response curve for bradykinin in β -arrestin- Ca^{2+} _{MPX}Nomad-BK2 cell line analyzed using the “Synergy 2” microplate reader (Biotek). **Left panel)** Concentration response curve for bradykinin for red arrestin biosensor. **Right panel)** Concentration response curve for bradykinin for green Ca^{2+} biosensor.