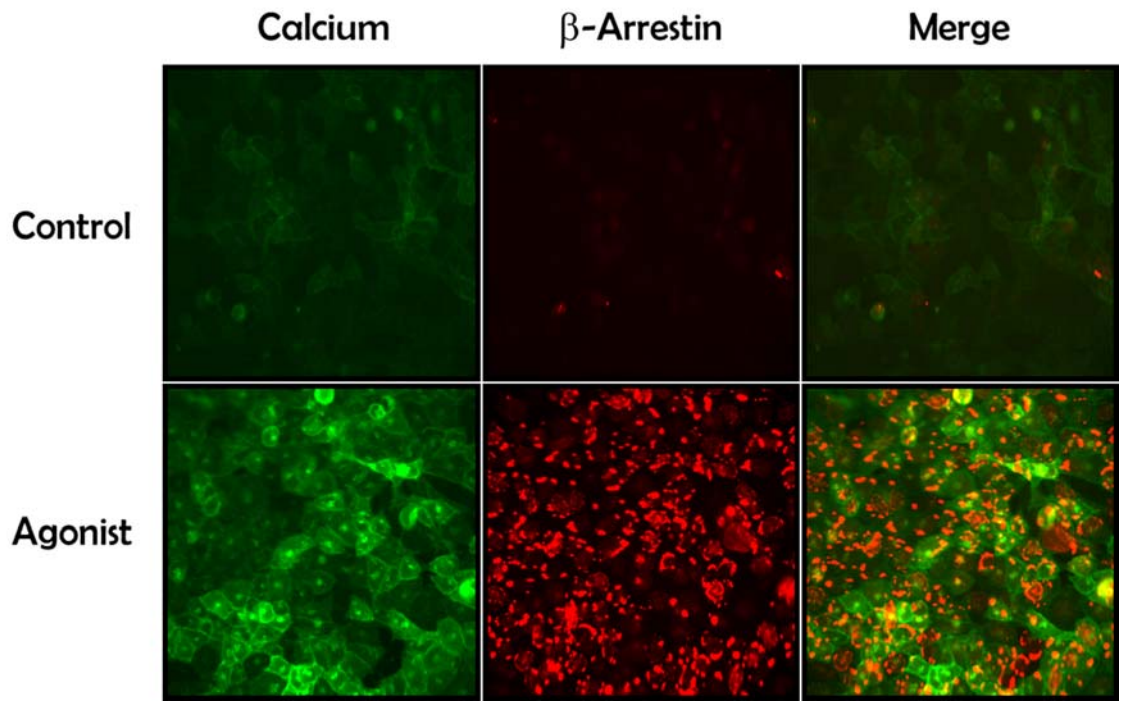
REF: P70723

NOMAD PAR2 CELL LINE

MULTIPLEX CELL LINES – Calcium and ß-Arrestin



MPXNomad-PAR2 (U2OS cell line)

Ec_{50} SLIGKV ß-Arrestin assay: $4.83x10^{-6}$ M

Ec_{50} SLIGRL-NH2 ß-Arrestin assay: $1.27x10^{-5}$ M

Ec_{50} SLIGKV calcium assay: $1.67x10^{-6}$ M

Ec_{50} SLIGRL-NH2 calcium assay: $4.06x10^{-6}$ M

Z´ SLIGKV $_{ß\text{-Arrestin}}$: 0.85+/- 0.01

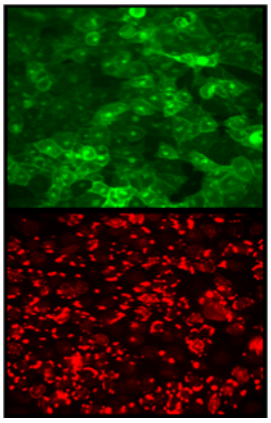
Z´ SLIGKRL-NH2 $_{ß\text{-Arrestin}}$: 0.75+/- 0.01

Z´ SLIGKV $_{Calcium}$: 0.68+/- 0.01

Z´ SLIGRL-NH2 $_{Calcium}$: 0.74+/- 0.01

VAT No. ESB95481909

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com



Product Name: PAR2 $_{MPX}$Nomad cell line (GPR11 / F2RL1)

Reference: P70723

Recp. Official Full Name: Protease activated receptor 2

DNA Accession Number: NM-005242.4

Host Cell: U2OS

Resistance: G418 + Puromycin + Hygromycin

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of $_{MPX}$Nomad-PAR2 contains U2OS cells stably expressing red $_{ß\text{-}Arrestin}$Nomad and green $_{Ca2+}$Nomad biosensor and Protease activated receptor 2 (with no tag).

Innoprot´s $_{MPX}$Nomad-PAR2 cell line has been designed to assay compounds or analyze their capability to modulate Protease activated receptor 2. When an agonist binds to PAR2 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 fkuorescence intensity increase within the cell.

This highly reproducible assay has been validated using human SLIGKV-NH2 and SLIGRL-NH2 as agonists in a High Content Analysis (HCA) and a High Throughput Analysis (HTA).

About Nomad Biosensor Family

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

Before the stimulation mediated by the agonist of interest, the fluorescent biosensor is localized in the cellular membrane. An increase in this second messenger concentration leads to a change in the structural folding of Nomad Biosensor that promotes its cellular relocation in the vesicular trafficking of the cells and an increase in the fluorescence.

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

VAT No. ESB95481909

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

ß-Arrestin and Calcium Functional Assays

ß-arrestin-Ca^{2+} $_{MPX}$Nomad U2OS cells, stably expressing Protease activated receptor 2 (PAR2), were stimulated with 11 log dilution series ranging from 0 to 300 µM of SLIGKV and SLIGRL-NH2 during 24h (n=5). % Activity was calculated relative to positive (100µM).

Fluorescence intensity analysis

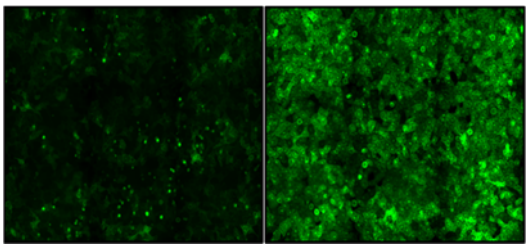


Fig1. ***Calcium biosensor fluorescence*** intensisty increase in $_{MPX}$Nomad PAR2 cell line stimulated with 10µM of SLIGKV.

The increase in the calcium biosensor fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The **EC50** for the SLIGKV was ~ **1.67x10^{-6}M** and **EC50** for the SLIGRL-NH2 was ~ **4.06 x10^{-6}M** after a treatment of 24 h with the agonists. The assay was validated with an average of **Z´= 0.85**+/-0.01 and **Z´= 0.75**+/-0.01 for SLIGKV and SLIGRL-NH2, respectively.

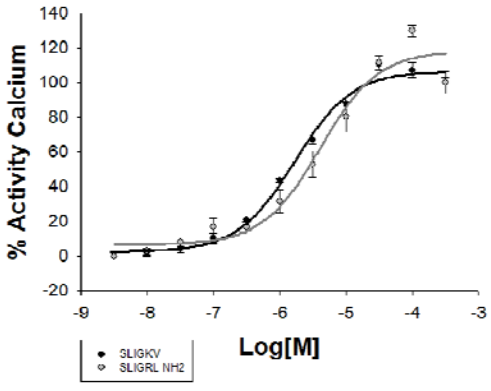


Fig2. Concentration response curve for SLIGKV and SLIGRL-NH2 in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-PAR2 cell line analyzed using a microplate reader.

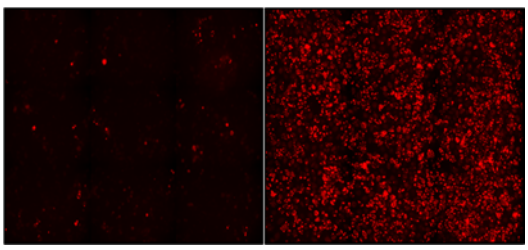


Fig3. ***ß-arrestin biosensor fluorescence*** intensisty increase in $_{MPX}$Nomad PAR2 cell line stimulated with 10µM of SLIGKV.

The increase in the ß-arrestin biosensor fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The **EC50** for the SLIGKV was~ **4.83x10^{-6}M** and **EC50** for the SLIGRL-NH2 was ~ **1.27 x10^{-5}M** after a treatment of 24 h with the agonists. The assay was validated with an average of **Z´= 0.68**+/-0.01 and **Z´= 0.74**+/-0.01 for SLIGKV and SLIGRL-NH2, respectively.

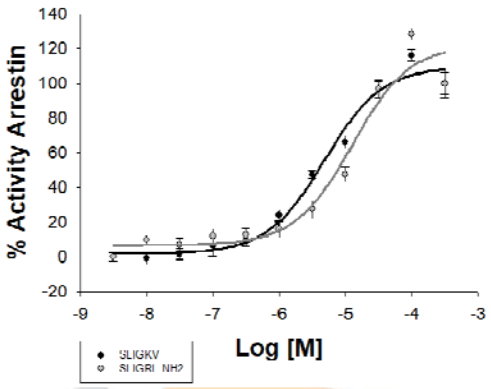


Fig4. Concentration response curve for SLIGKV and SLIGRL-NH2 in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-PAR2 cell line analyzed using a microplate reader.

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