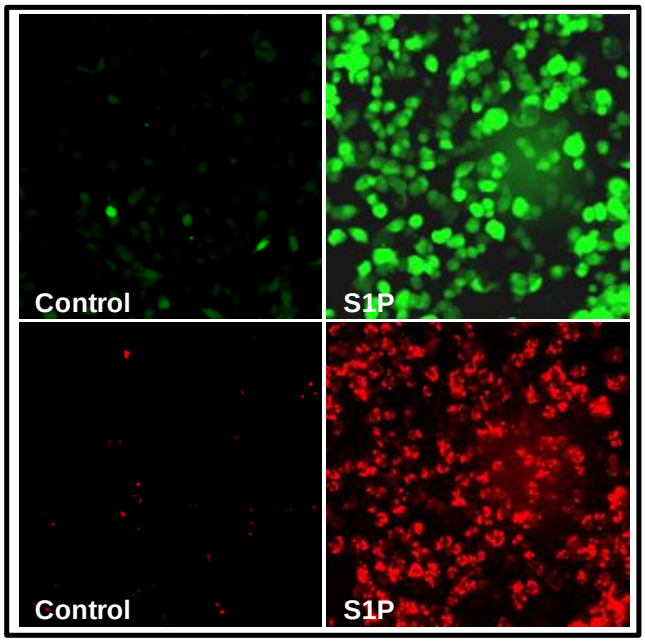
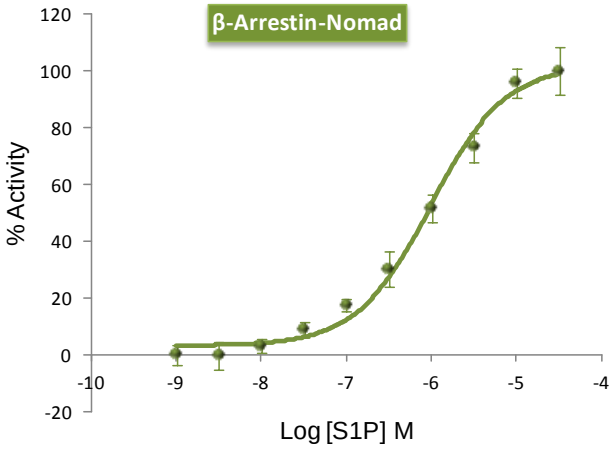
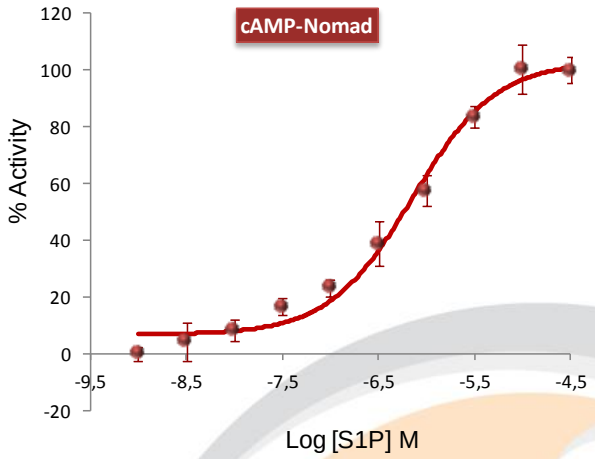
MULTIPLEX CELL LINES –ß-Arrestin and cAMP
MPXNOMAD SPHINGOSINE-1-PHOSPHATE RECEPTOR 3 (S1P3R)





EC_{50} ß-Arrestin assay: $9.3x10^{-7}$ M

$Z'_{ß\text{-}Arrestin}$: 0.65



EC_{50} cAMP assay: $7.14x10^{-7}$ M

Z'_{cAMP}: 0.79

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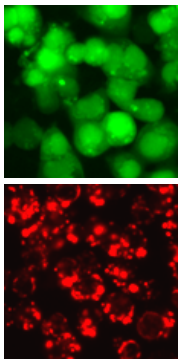

Product Name: $_{MPX}$Nomad-S1P3R cell line

Reference: P70785

Receptor Official Full Name: Sphingosine-1-phosphate receptor 3

Host Cell: HEK293

Resistance: Puromycin + G418 + Hygromycin

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of $_{MPX}$Nomad-S1P3R contains HEK293 cells stably expressing green $_{ß\text{-}Arrestin}$Nomad, red $_{cAMP}$Nomad biosensor and sphingosine-1-phosphate receptor 3 (with no tag).

Innoprot´s $_{MPX}$Nomad-S1P3R cell line has been designed to assay compounds or analyze their capability to modulate sphingosine-1-phosphate receptor 3. When an agonist binds to S1P3R a G protein is activated which, in turn, triggers a cellular response mediated by cAMP and a subsequent internalization mediated by ß-Arrestin.

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

This highly reproducible assay has been validated using S1P as agonist in 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 - cAMP) 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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MULTIPLEX CELL LINES – cAMP and ß-Arrestin

ß-arrestin-cAMP $_{MPX}$Nomad HEK293 cells, stably expressing sphingosine-1-phosphate receptor 3 (S1P3R), were stimulated with increasing concentrations ranging from 0 to 30 µM of S1P during 24h (n=4) (Fig 1). The data were normalized as percentages of activity compared with the positive control (S1P) after subtracting the value of the vehicle control.

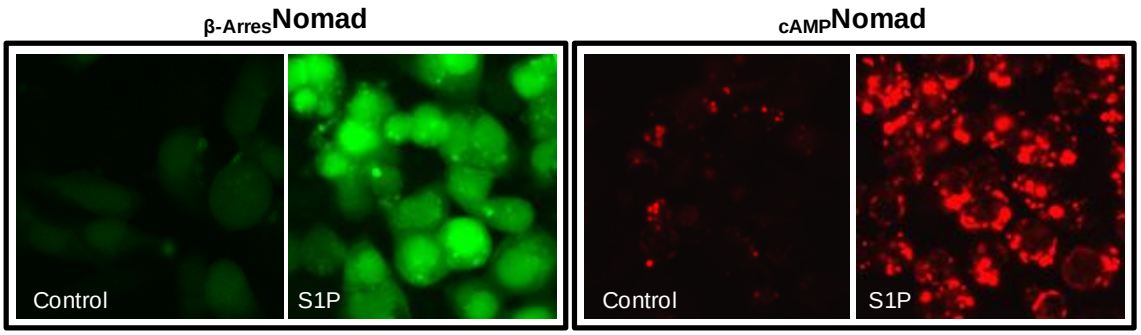

Fig 1. cAMP-ß-arrestin $_{MPX}$Nomad biosensor stimulated with 30 µM of S1P. *Left (green)*: $_{\beta Arr}$Nomad; *Right* (*red)*: $_{cAMP}$Nomad.

The increase in the fluorescence was detected and analyzed using the "Synergy 2" microplate reader from Biotek. The EC_{50} for S1P after a treatment of 24 h was 9.3 x10^{-7} M for the ß-arrestin assay (Z´ factor= 0.65) and 7.14x10^{-7} M for the cAMP assay (Z´ factor=0.79) (Fig 2).

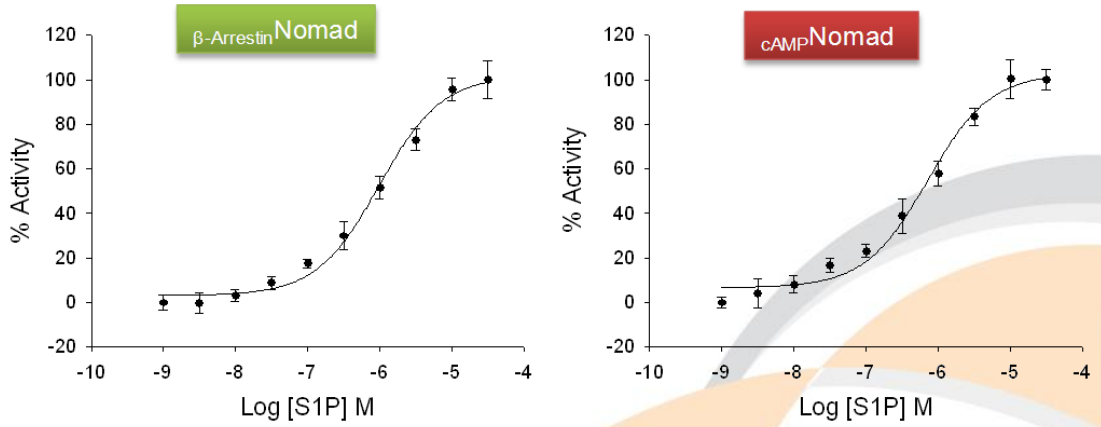

Fig 2. Concentration-response curve for S1P in ß-arrestin-cAMP $_{MPX}$Nomad-S1P3R cell line analyzed using the "Synergy 2" microplate reader (Biotek). **Left panel)** Concentration response curve for S1P for green arrestin biosensor. **Right panel)** Concentration response curve for S1P for red cAMP biosensor.

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