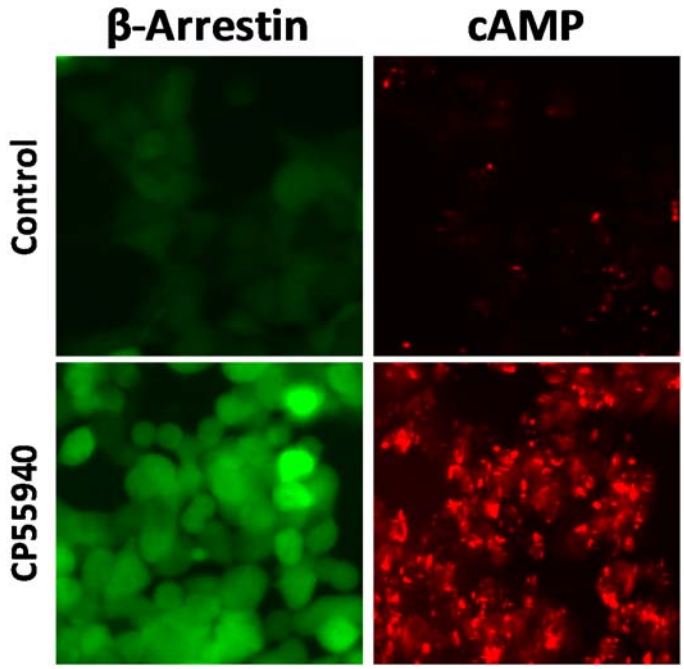
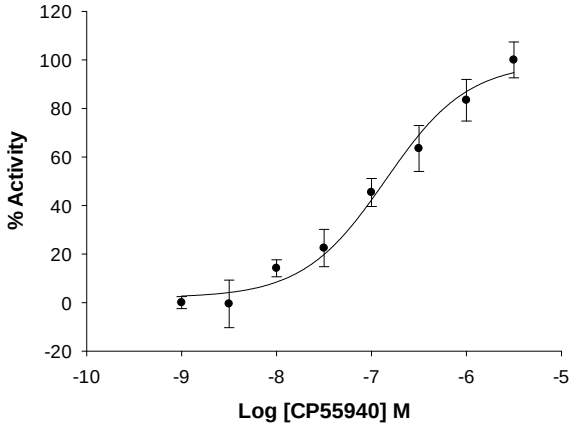
REF: P70727

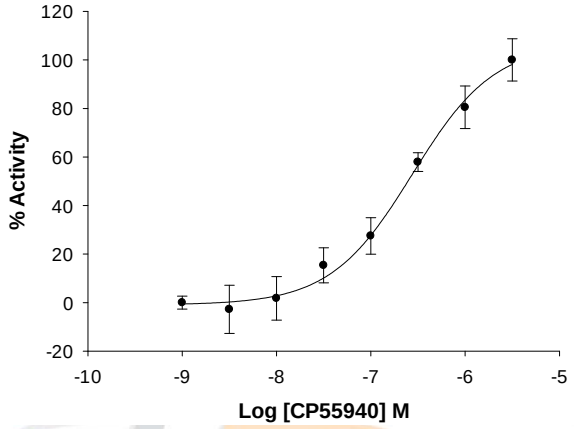
MULTIPLEX CELL LINES – cAMP and ß-Arrestin

MPXNOMAD CANNABINOID RECEPTOR 1 (CB1)

MPXNomad-CB1 (HEK293 cell line)







EC_{50} ß-Arrestin assay: 1.4 x 10^{-7} M

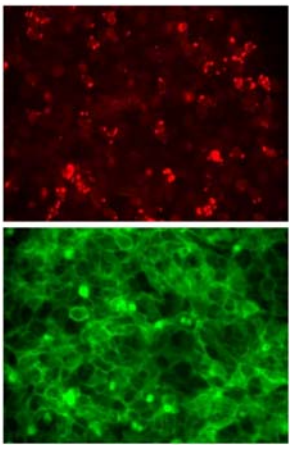
$Z'_{ß\text{-Arrestin}}$: 0.72

EC_{50} cAMP assay: 2.25 x 10^{-7} M

$Z'_{Calcium}$: 0.69

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: $_{MPX}$Nomad-CB1 cell line

Reference: P70727

Receptor Official Full Name: Cannabinoid receptor 1

DNA Accession Number: AY225225.1

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-CB1 contains HEK293 cells stably expressing both green $_{ß\text{-}Arrestin}$Nomad and red $_{cAMP}$Nomad biosensors and Cannabinoid receptor 1 (with no tag).

Innoprot´s $_{MPX}$Nomad-CB1 cell line has been designed to assay compounds or analyze their capability to modulate Cannabinoid receptor 1. When an agonist binds to CB1 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 signalling and ß-Arrestin mobilization analyzing Nomad biosensors fluorescence intensity in living cells.

This highly reproducible assay has been validated using CP55940 as agonist in a High Throughput Screening (HTS).

About Nomad Biosensor Family

Nomad Biosensor family is based in a fluorescent polypeptide that measures fluctuations in the cAMP 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 (cAMP + β-arrestin) 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 cAMP Assay

ß-arrestin-cAMP $_{MPX}$Nomad HEK293 cells, stably expressing Cannabinoid receptor 1 (CB1), were stimulated with 8 dilution series ranging from 0 to 3 µM of CP55940 during 24h (n=4). % Activity was calculated relative to positive.

Fluorescence intensity analysis

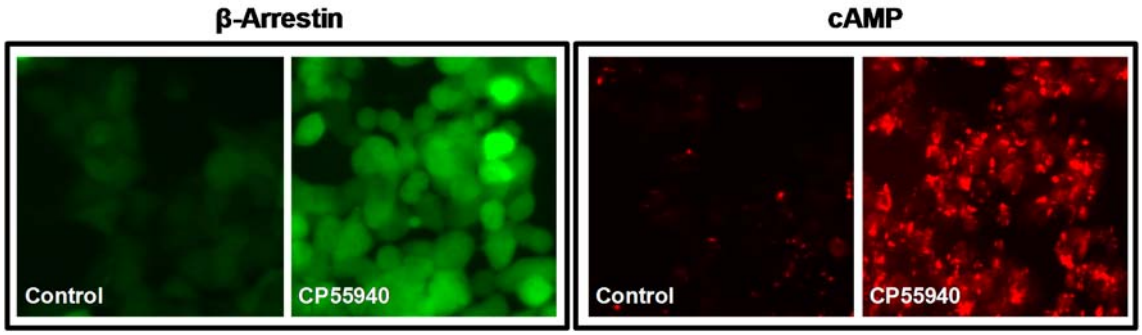


Fig 1. ß-arrestin-cAMP $_{MPX}$Nomad biosensor stimulated with 3 µM of CP55940. *Left (green)*: ß-arrestin biosensor; *Right* (*red)*: cAMP biosensor.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC_{50} for CP55940 after a treatment of 24 h was 1.4 x10^{-7} M for the ß-arrestin assay (validated with a Z´= 0.72) and 2,25x10-7M for the calcium assay (Z´=0.69).

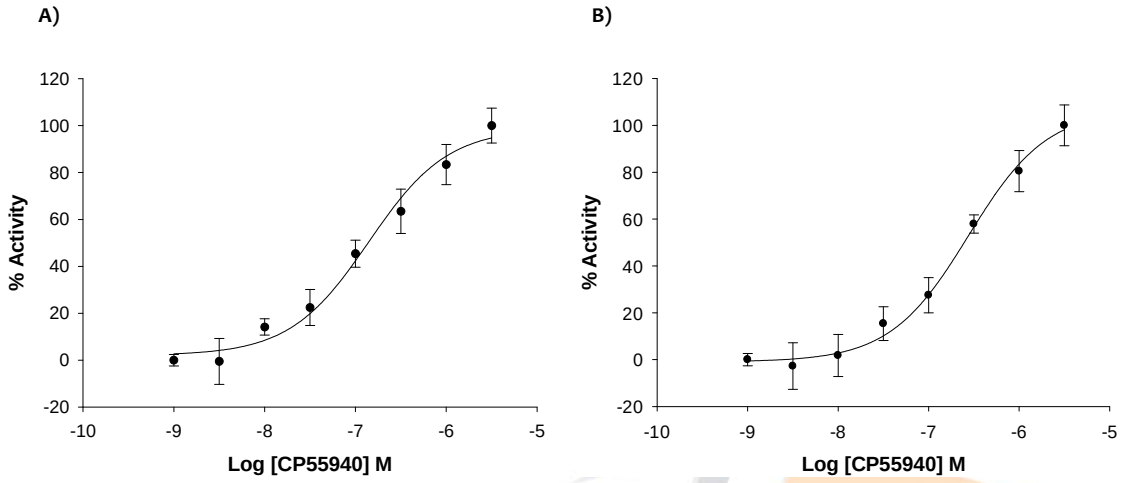


Fig 2. Concentration-response curve for CP55940 in ß-arrestin-cAMP $_{MPX}$Nomad-CB1 cell line analyzed using the "Synergy 2" microplate reader (Biotek). **A)** Concentration response curve for CP55940 for green arrestin biosensor. **B)** Concentration response curve for CP55940 for red calcium biosensor.

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