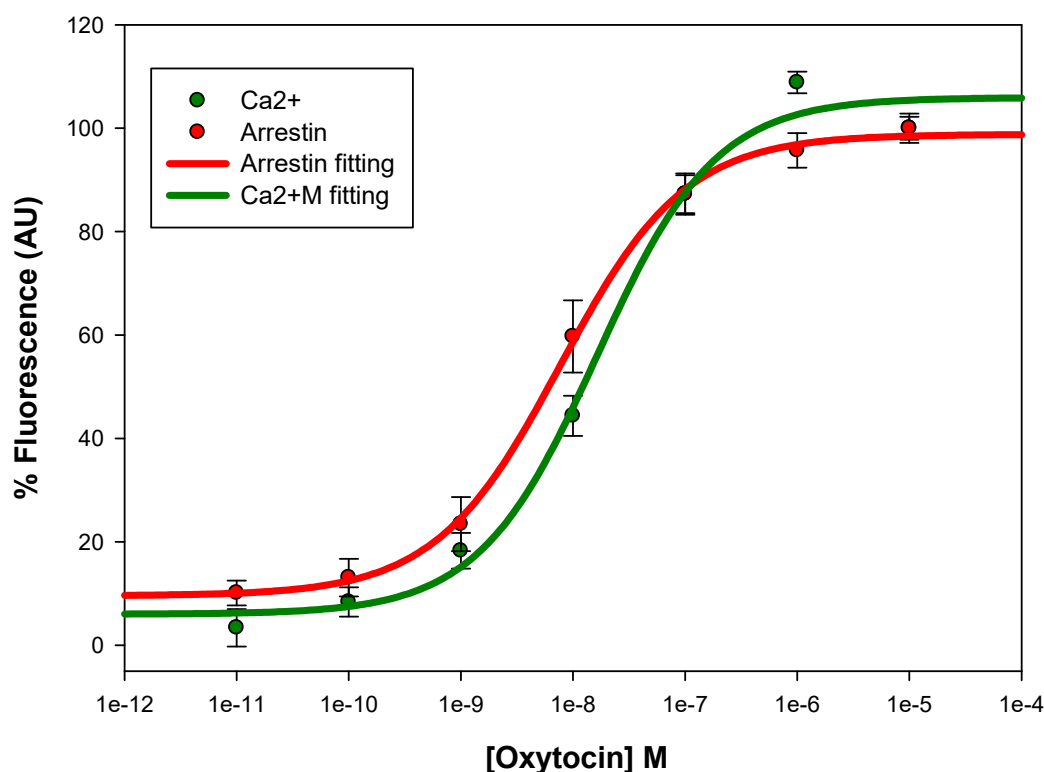


MULTIPLEX CELL LINES – Calcium and β -Arrestin

MPXNOMAD OXYTOCIN RECEPTOR (OT RECEPTOR)

OTR



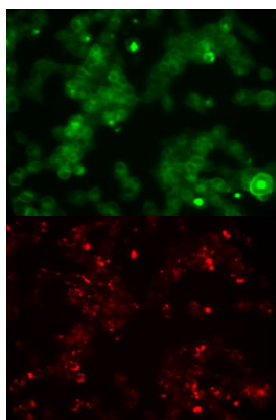
MPXNomad-OTR (HEK293 cell line)

Ec₅₀ β -Arrestin1 assay: 7.72×10^{-9} M

Ec₅₀ calcium assay: 1.63×10^{-8} M

Z' β -Arrestin1: 0.72

Z' Calcium: 0.80



Product Name: OTR_{MPX}Nomad cell line

Reference: P70725

Recp. Official Full Name: Oxytocin Receptor 2

DNA Accession Number: Gene Bank NP_000907.2

Host Cell: HEK293

Resistance: G418 + Puromycin

Quantity: > 3 x 10⁶ cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of MPXNomad-OTR contains HEK293 cells stably expressing red β -Arrestin_{Nomad} and green Ca^{2+} _{Nomad} biosensor and Oxytocin (OT) receptor (with no tag). Innoprot's MPXNomad-OTR cell line has been designed to assay compounds or analyze their capability to modulate OT receptor. When an agonist binds to OT receptor 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 human Neurotensin as agonist 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 localization and fluorescence 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 the 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 MPXNomad Biosensor and a GPCR, the activity can be easily quantified on living cells by image analysis or fluorescence emission in a microplate reader.

β -Arrestin and Calcium Assays

β -arrestin- Ca^{2+} MPXNomad HEK293 cells, stably expressing Oxytocin receptor (OTR), were stimulated with 7 log dilution series ranging from 0 to $10\mu\text{M}$ of oxytocin during 24h (n=5). % Activity was calculated relative to positive ($1\mu\text{M}$).

Fluorescence intensity analysis

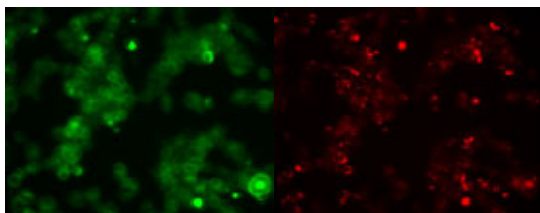


Fig1. β -arrestin- Ca^{2+} MPXNomad biosensor stimulated with $1\mu\text{M}$ of OT (Green; Ca^{2+} biosensor, Red; Arrestin biosensor).

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC50 after a treatment of 24 h with the agonist for β -arrestin assay was $\sim 7.72 \times 10^{-9}\text{M}$ validated with $Z' = 0.72$ and for calcium assay the EC50 was $\sim 1.63 \times 10^{-8}\text{M}$ and $Z' = 0.80$.

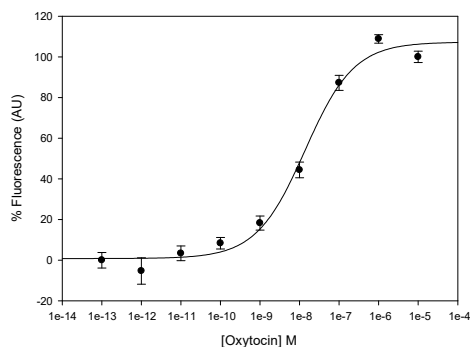


Fig2. Concentration response curve for OT for green Ca^{2+} biosensor, in β -arrestin- Ca^{2+} MPXNomad-OTR cell line analyzed using a microplate reader.

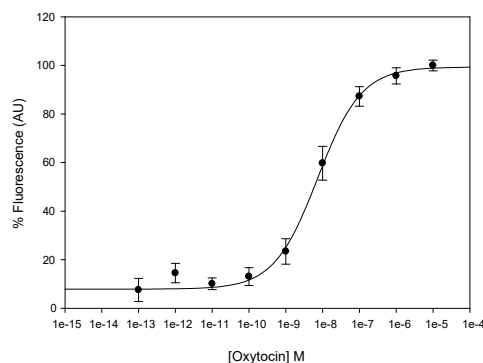


Fig3. Concentration response curve for OT for red Arrestin biosensor, in β -arrestin- Ca^{2+} MPXNomad-OTR cell line analyzed using a microplate reader.