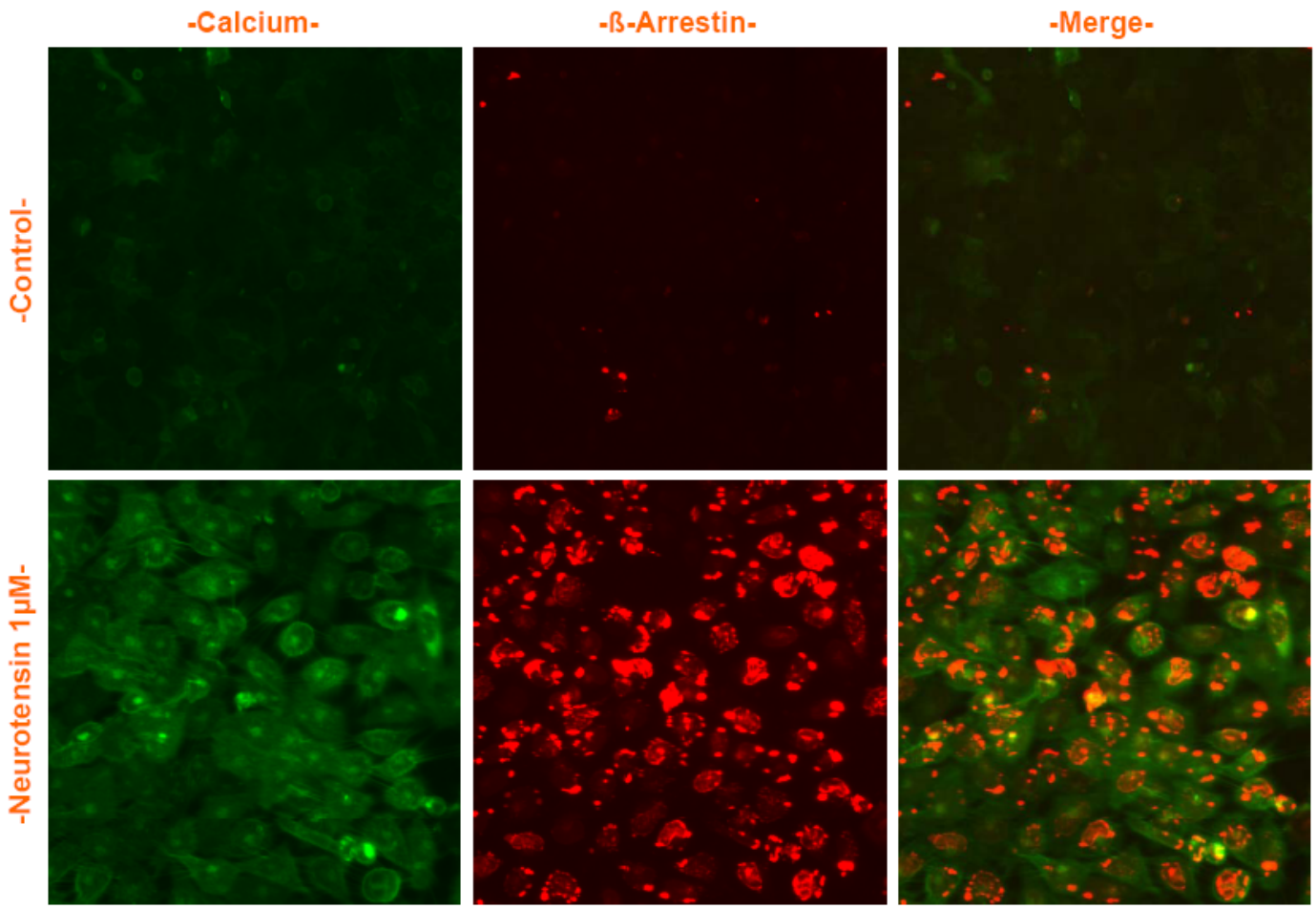
REF: P70746

MULTIPLEX CELL LINES – Calcium and ß-Arrestin

MPXNOMAD NEUROTENSIN RECEPTOR 1



MPXNomad-NTSR1 (U2OS cell line)

Ec_{50} ß-Arrestin1 assay: $7.67x10^{-9}$ M

Ec_{50} calcium assay: $3.75x10^{-9}$ M

$Z'_{ß\text{-}Arrestin1}$: 0.81+/- 0.01

$Z'_{Calcium}$: 0.74+/- 0.01

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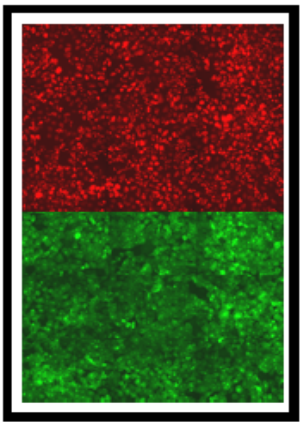

Product Name: NTSR1 $_{MPX}$Nomad cell line

Reference: P70746

Recp. Official Full Name: Neurotensin Receptor1

DNA Accession Number: AY429106

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-NTSR1 contains U2OS cells stably expressing red $_{ß\text{-}Arrestin}$Nomad and green $_{Ca2+}$Nomad biosensor and Neurotensin 1 receptor (with no tag).

Innoprot´s $_{MPX}$Nomad-NTSR1 cell line has been designed to assay compounds or analyze their capability to modulate Neurotensin receptor 1. When an agonist binds to NTSR1 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 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 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 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 $_{MPX}$Nomad Biosensor and a GPCR, the activity can be easily quantified on living cells by image analysis or fluorescence emission in a microplate reader.

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ß-Arrestin and Calcium Assay

ß-arrestin-Ca^{2+} $_{MPX}$Nomad U2OS cells, stably expressing Neurotensin 1 receptor (NTSR1), were stimulated with 7 log dilution series ranging from 0 to 1 µM of Neurotensin during 24h (n=5). % Activity was calculated relative to positive (1µM).

Image analysis

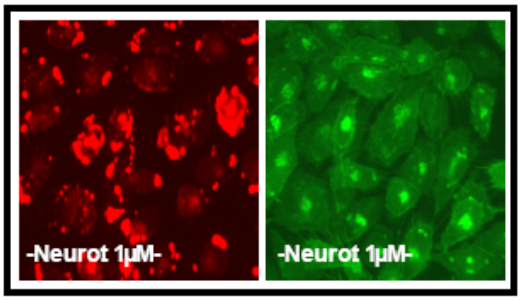

Fig1. ß-arrestin-Ca^{2+} $_{MPX}$Nomad biosensor stimulated with 1µM of Neurotensin.

Biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The EC50 after a treatment of 24 h with the agonist for ß-arrestin assay was ~ 7.67x10^{-9}M validated with an average of Z´= 0.89+/-0.02 and for calcium assay the Ec50 was ~ 3.75x10^{-9}M and Z´=0.74

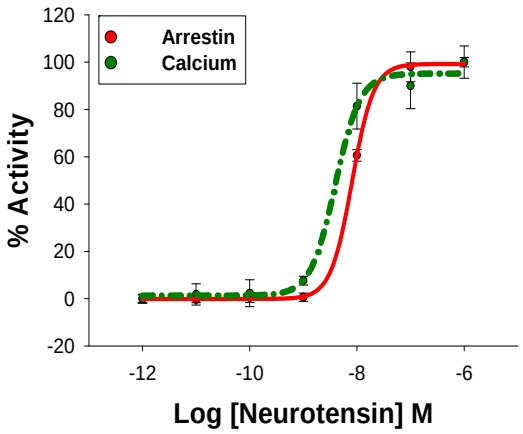

Fig2. Concentration response curve for Neurotensin in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-NTSR1 cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

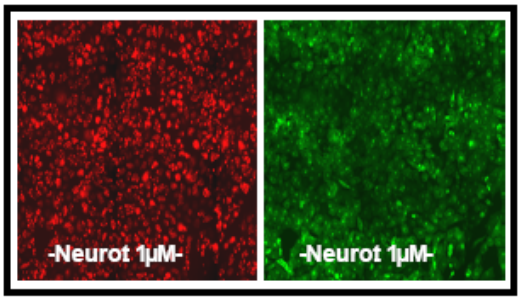

Fig3. ß-arrestin-Ca^{2+} $_{MPX}$Nomad biosensor stimulated with 1µM of Neurotensin.

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 ß-arrestin assay was ~ 7.74x10^{-9}M validated with an average of Z´= 0.90+/-0.02 and for calcium assay the Ec50 was ~ 3.38x10^{-9}M and Z´=0.84

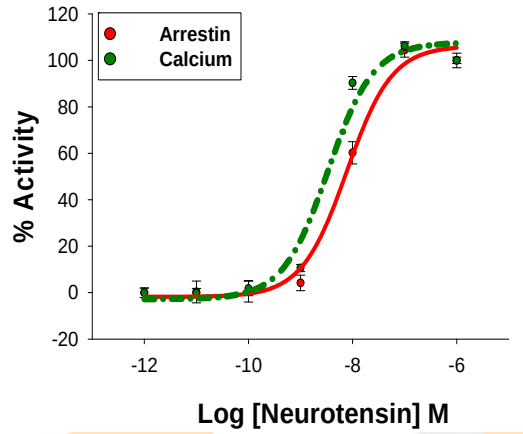

Fig4. Concentration response curve for Neurotensin in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-NTSR1 cell line analyzed using a microplate reader.

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ß-Arrestin and Calcium Antagonism Assay

ß-arrestin-Ca^{2+} $_{MPX}$Nomad U2OS cells, stably expressing Neurotensin 1 receptor (NTSR1), were co-treated with 8 log dilution series ranging from 0 to 3 µM of SR48692 and Neurotensin 10 nM during 24h (n=5) for antagonism assay. % Activity was calculated relative to negative control.

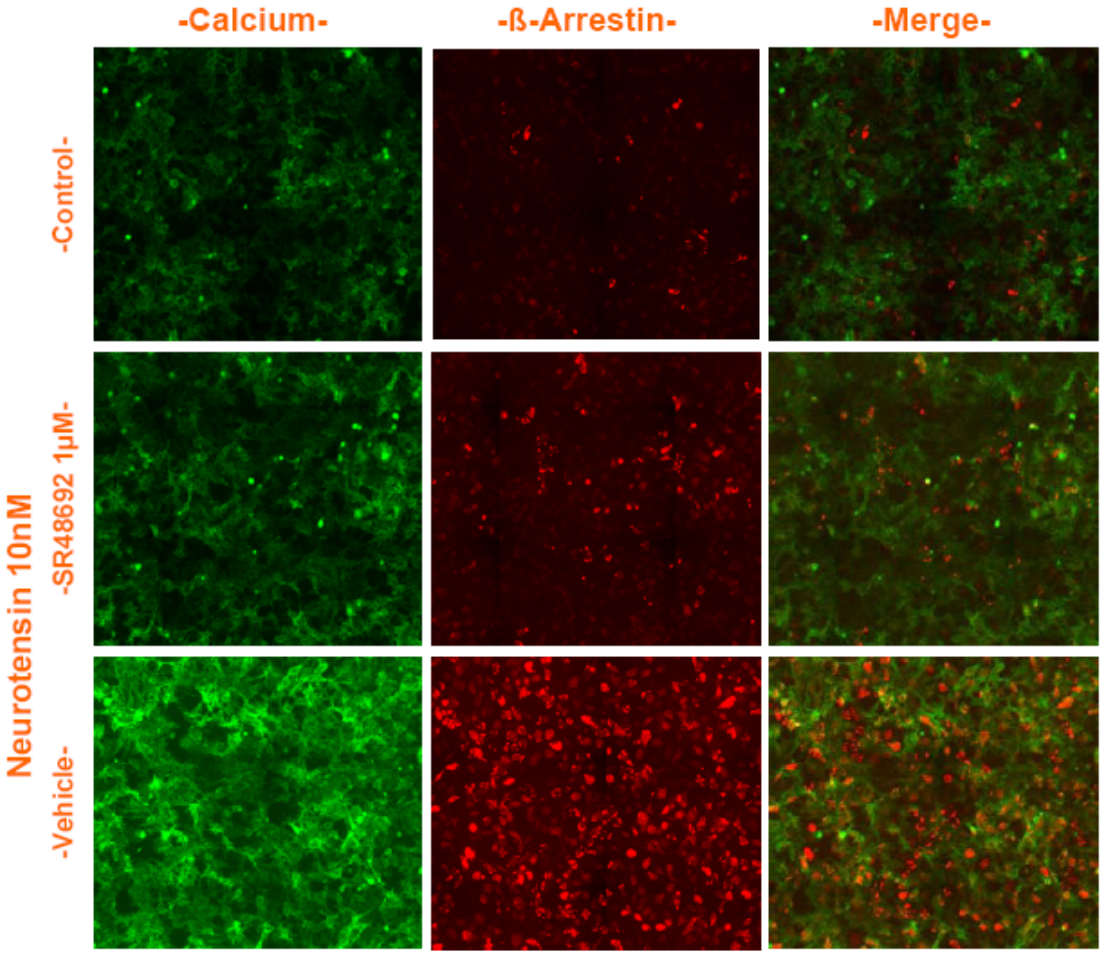

Fig5. ß-arrestin-Ca^{2+} $_{MPX}$Nomad biosensor cotreated with 10nM of Neurotensin and 3µM of SR48692.

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Image analysis

Biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The IC50 after a treatment of 24 h with the antagonist for ß-arrestin assay was ~ $5.33x10^{-8}$M validated with an average of Z´= 0.58+/-0.02 and for calcium assay the Ic50 was ~ $1.25x10^{-7}$M and Z´=0.52

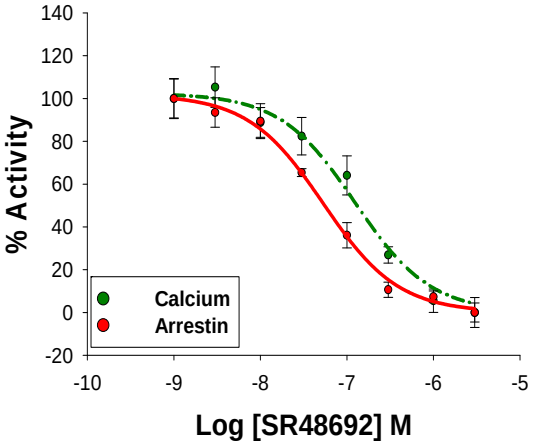

Fig6. Concentration response curve for SR48692 in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-NTSR1 cell line cotreated with Neurotensin 10 nM and analyzed using a high-content bioimager.

Fluorescence intensity analysis

Changes in the fluorescence intensity were detected and analyzed using "Synergy 2" microplate reader from Biotek. The IC50 after a treatment of 24 h with the agonist for ß-arrestin assay was ~ $1.42x10^{-7}$M validated with an average of Z´= 0.63+/-0.02 and for calcium assay the Ec50 was ~ $1.08x10^{-7}$M and Z´=0.68

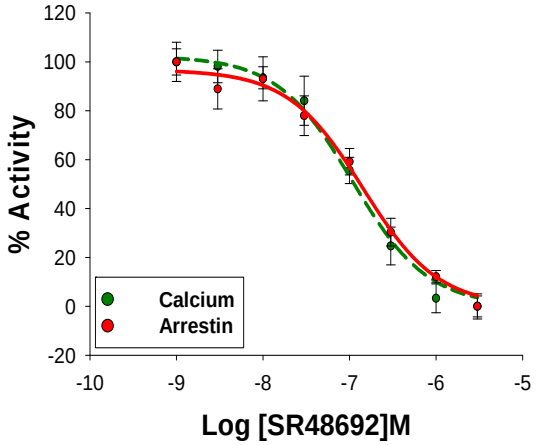

Fig7. Concentration response curve for SR48692 in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-NTSR1 cell line cotreated with Neurotensin 10 nM and analyzed using a microplate reader.

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