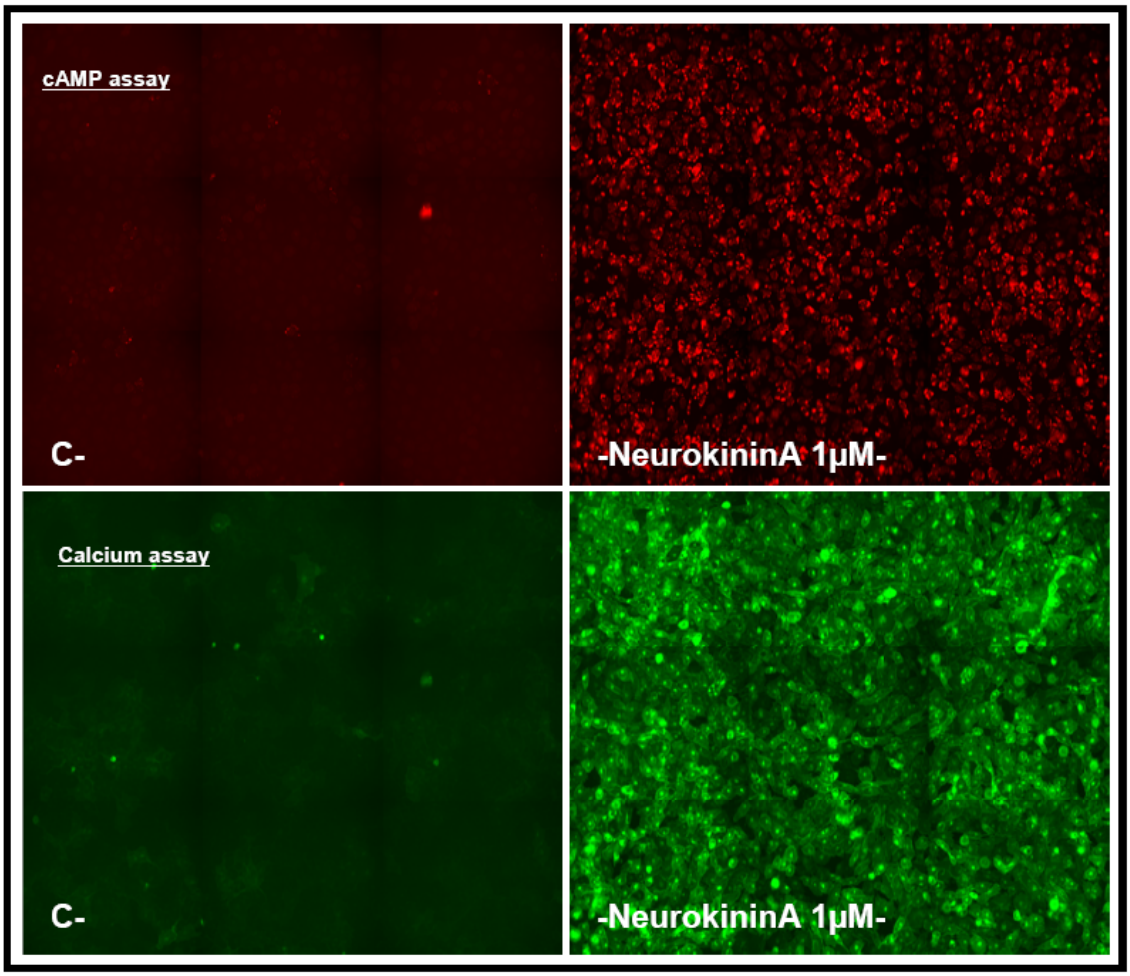
REF: P70756

MULTIPLEX CELL LINES – cAMP and Calcium ASSAY

-NEUROKININ 2 RECEPTOR (TACR2)-



Multiplexed Nomad TACR2 (U2OS cell line)

Ec_{50} cAMP assay: $5.61x10^{-9}$ M

Ec_{50} calcium assay: $2.38x10^{-9}$ M

Z'_{cAMP}: 0.85+/- 0.01

$Z'_{Calcium}$: 0.81+/- 0.01

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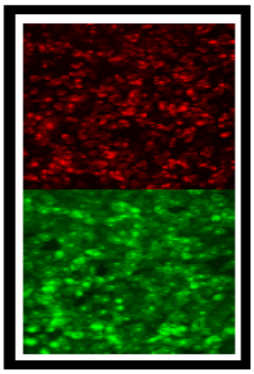

Product Name: TACR2 Multiplexed Nomad cell line
Reference: P70756
Recp. Official Full Name: Neurokinin receptor 2
DNA Accession Number: AY322545
Host Cell: U2OS
Resistance: G418 + Puromycin + Hygromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of Multiplexed Nomad TACR2 contains U2OS cells stably expressing cAMP Nomad-FP650 biosensor, Calcium Nomad-tGFP biosensor and Neurokinin receptor 2 (with no tag).

Innoprot Multiplexed Nomad TACR2 cell line has been designed to assay compounds or analyze their capability to modulate Neurokinin receptor 2. When an agonist binds to TACR2 a G protein is activated, which in turn, triggers a cellular response mediated by cAMP and calcium.

This cell line has been validated measuring cAMP and calcium increase in the cytosol analyzing cAMP and calcium biosensors distribution within the cell.

This highly reproducible assay has been validated using Neurokinin A 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 in the presence or absence of cAMP or calcium changes its localization 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 (cAMP) or an increase in the fluorescence (calcium).

In a cell line co-expressing Multiplexed 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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cAMP Assay

Multiplexed Nomad U2OS cells, stably expressing Neurokinin receptor 2 (TACR2), were stimulated with 7 log dilution series ranging from 0 to 1 µM of Neurokinin A during 24h (n=5). % Activity was calculated relative to positive (1µM).

Image analysis

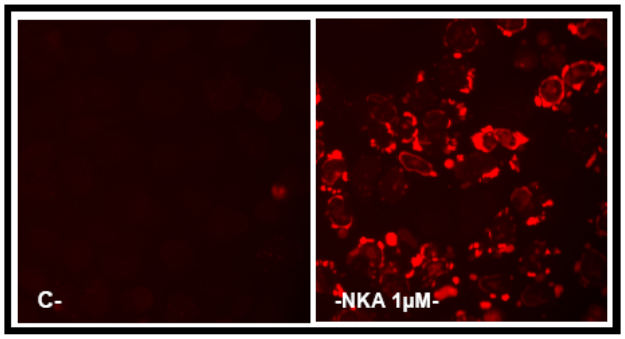

Fig1. Red Nomad-cAMP biosensor negative control and Neurokinin A stimulation.

Activation and biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The **EC50** for the Neurokinin A was ~ **$9.91x10^{-9}$M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.81**+/-0.02.

Fluorescence intensity analysis

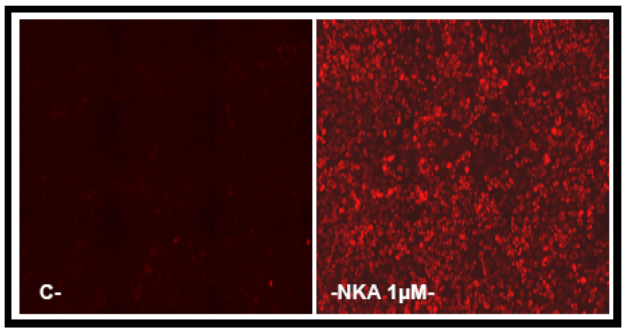

Fig2. Red Nomad-cAMP biosensor negative control and Neurokinin A stimulation.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The **EC50** for the Neurokinin A was ~ **$1.14x10^{-8}$M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.66**+/-0.02.

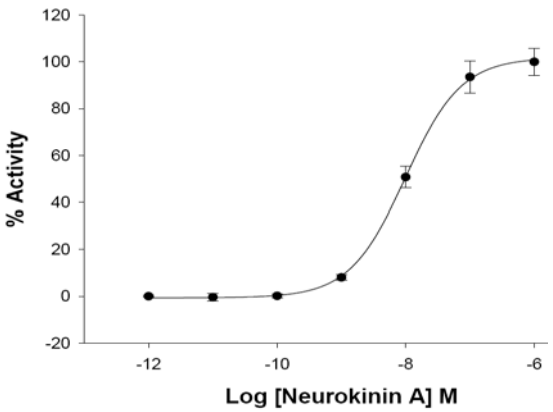

Fig3. Concentration response curve for Neurokinin A in Multiplexed Nomad TACR2 cell line analyzed using a high-content bioimager.

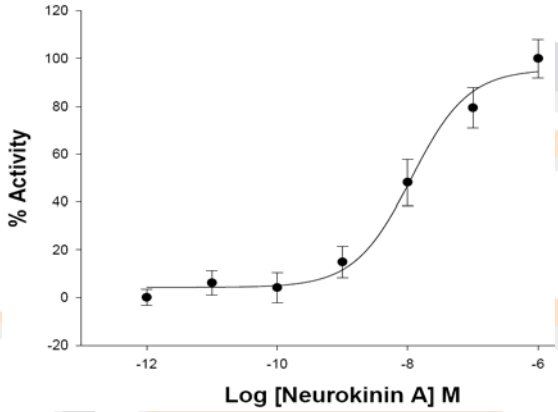

Fig4. Concentration response curve for Neurokinin A in Multiplexed Nomad TACR2 cell line analyzed using a fluorescence microplate reader.

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Calcium Assay

Multiplexed Nomad U2OS cells, stably expressing Neurokinin receptor 2 (TACR2), were stimulated with 7 log dilution series ranging from 0 to 1 µM of Neurokinin A during 24h (n=5). % Activity was calculated relative to positive (1µM).

Image analysis

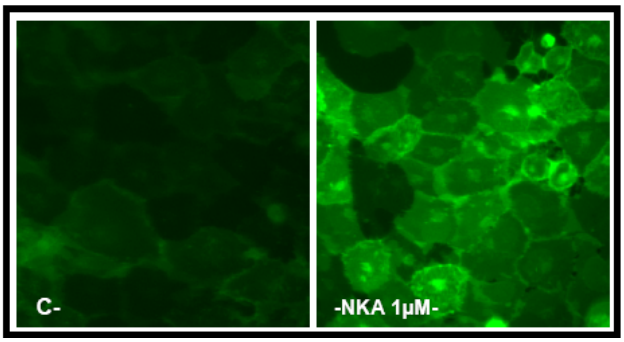

Fig5. Green Nomad-calcium biosensor negative control and Neurokinin A stimulation.

Fluorescence intensity analysis

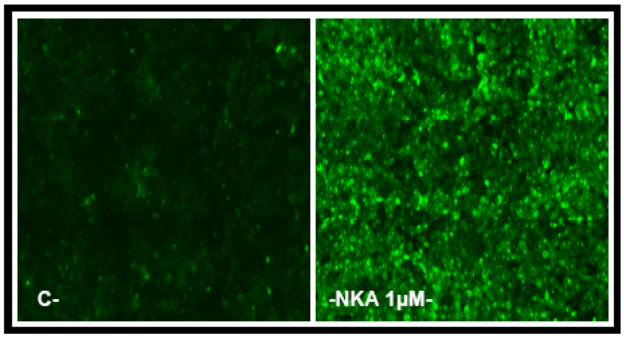

Fig6. Green Nomad-calcium biosensor negative control and Neurokinin A stimulation.

Activation and biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The **EC50** for the Neurokinin A was ~ **1.16x10^{-8}M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.60**+/-0.02.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The **EC50** for the Neurokinin A was ~ **6.15x10^{-9}M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.71**+/-0.02.

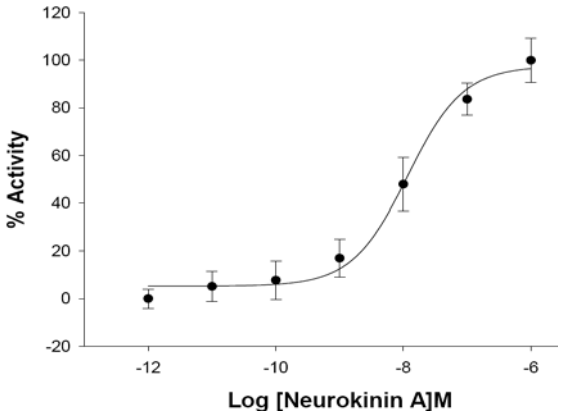

Fig7. Concentration response curve for Neurokinin A in Multiplexed Nomad TACR2 cell line analyzed using a high-content bioimager.

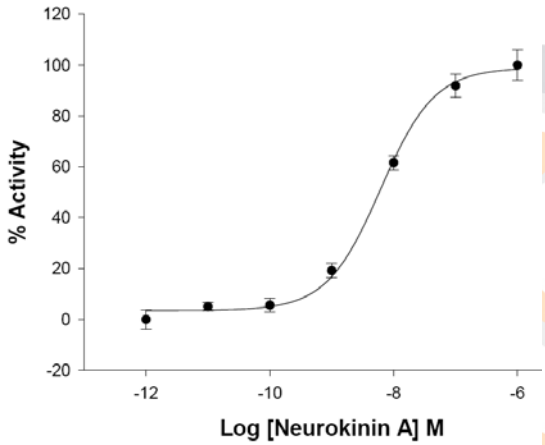

Fig8. Concentration response curve for Neurokinin A in Multiplexed Nomad TACR2 cell line analyzed using a fluorescence microplate reader.

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