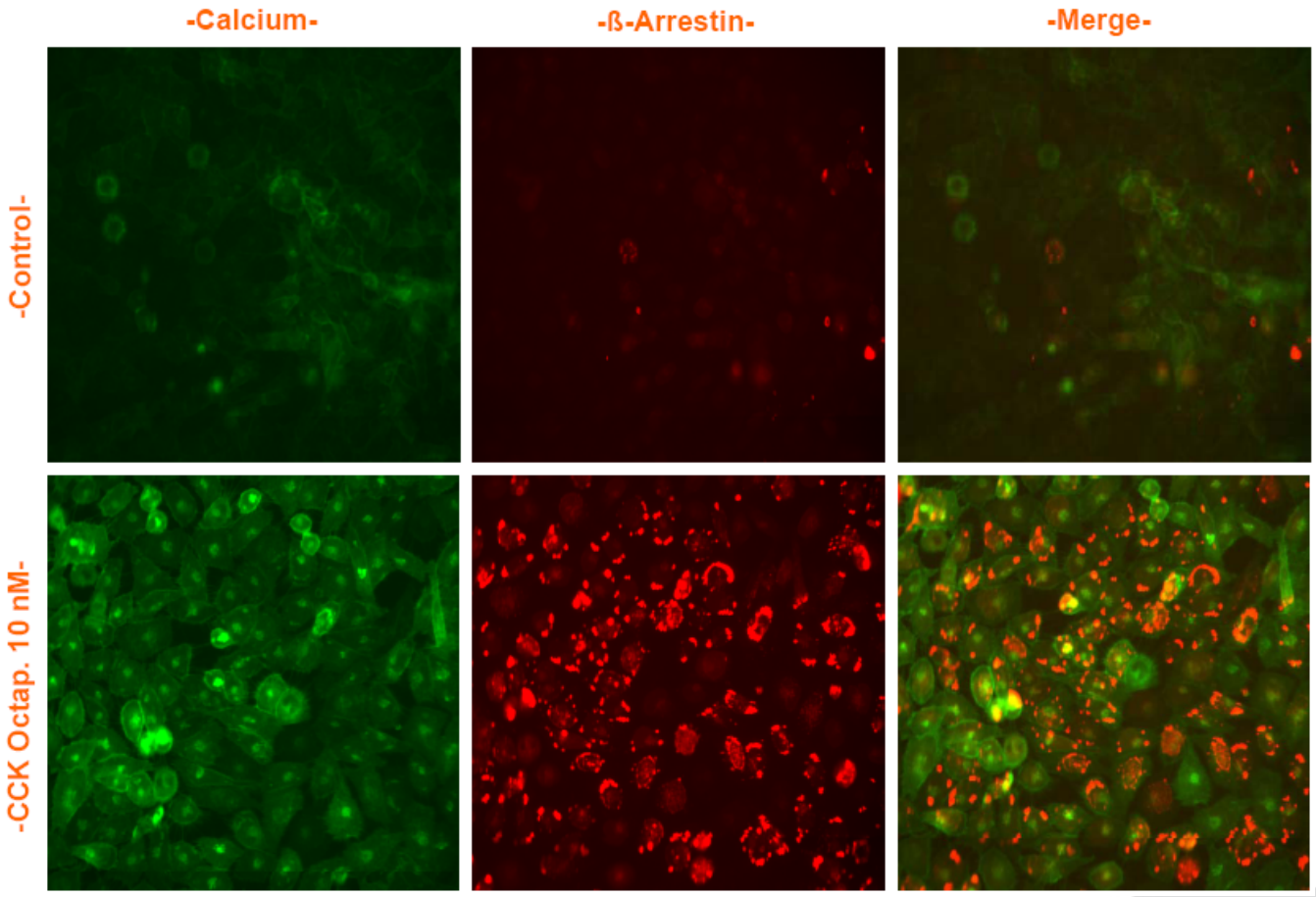
REF: P70708

MULTIPLEX CELL LINES – Calcium and ß-Arrestin

MPXNOMAD CCK$_2$ RECEPTOR



MPXNomad-CCK2R (U2OS cell line)

Ec$_{50}$ ß-Arrestin1 assay: 3.10x10^{-12} M **Ec$_{50}$ calcium assay:** 1.17 x 10^{-12} M

Z´$_{ß\text{-}Arrestin1}$: 0.71+/- 0.02 **Z´$_{Calcium}$:** 0.71+/- 0.01

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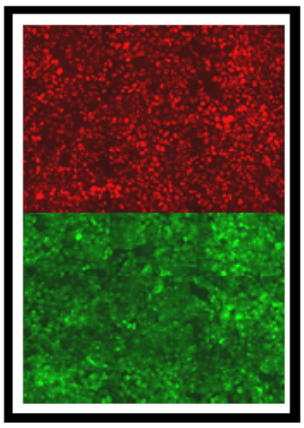

Product Name: CCK2R $_{MPX}$Nomad cell line
Reference: P70708
Recp. Official Full Name: Cholecystokinin 2 receptor
DNA Accession Number: AY322551
Host Cell: U2OS
Resistance: G418 + Puromycin + Hygromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of $_{MPX}$Nomad-CCK$_2$R 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-CCK$_2$R cell line has been designed to assay compounds or analyze their capability to modulate Neurotensin receptor 1. When an agonist binds to CCK$_2$R 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 fluorescence intensity of Nomad biosensors.

This highly reproducible assay has been validated using CCK Octapeptide as agonist in both High Troughput Screening (HTS) and High Content Analysis (HCA).

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 Biosensors 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 Cholecystokinin 2 receptor (CCK_2R), were stimulated with 9 log dilution series ranging from 0 to 10 nM of CCK Octapeptide during 24h (n=5). % Activity was calculated relative to positive (10nM).

Image analysis

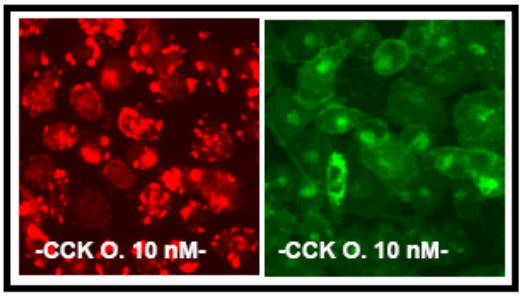

Fig1. ß-arrestin-$Ca^{2+}$$_{MPX}$Nomad biosensor stimulated with 10nM of CCK Octapeptide.

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 ~ $3.49x10^{-12}$M validated with an average of Z´= 0.62+/-0.02 and for calcium assay the Ec50 was ~ $9.66x10^{-13}$M and Z´=0.70

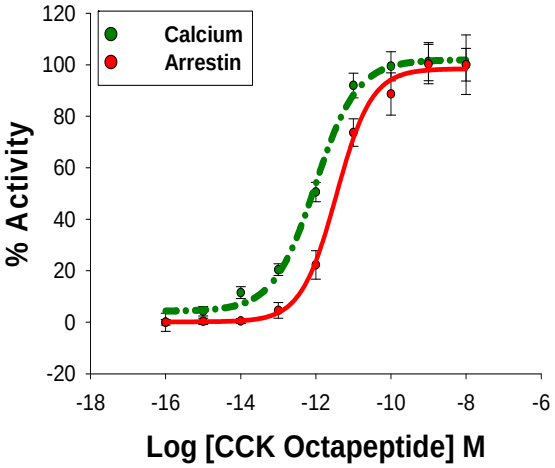

Fig2. Concentration response curve for CCK Oct. in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-CCK_2R cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

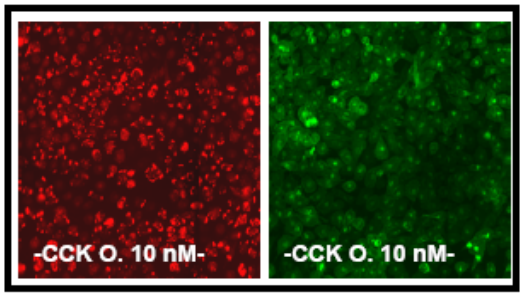

Fig3. ß-arrestin-$Ca^{2+}$$_{MPX}$Nomad biosensor stimulated with 10nM of CCK Octapeptide.

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 ~ $3.10x10^{-12}$M validated with an average of Z´= 0.71+/-0.02 and for calcium assay the Ec50 was ~ $1.17x10^{-12}$M and Z´=0.71

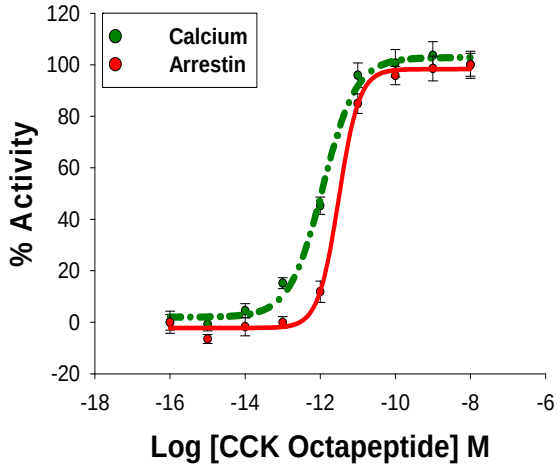

Fig4. C Concentration response curve for CCK Oct. in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-CCK_2R cell line analyzed using a microplate reader.

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