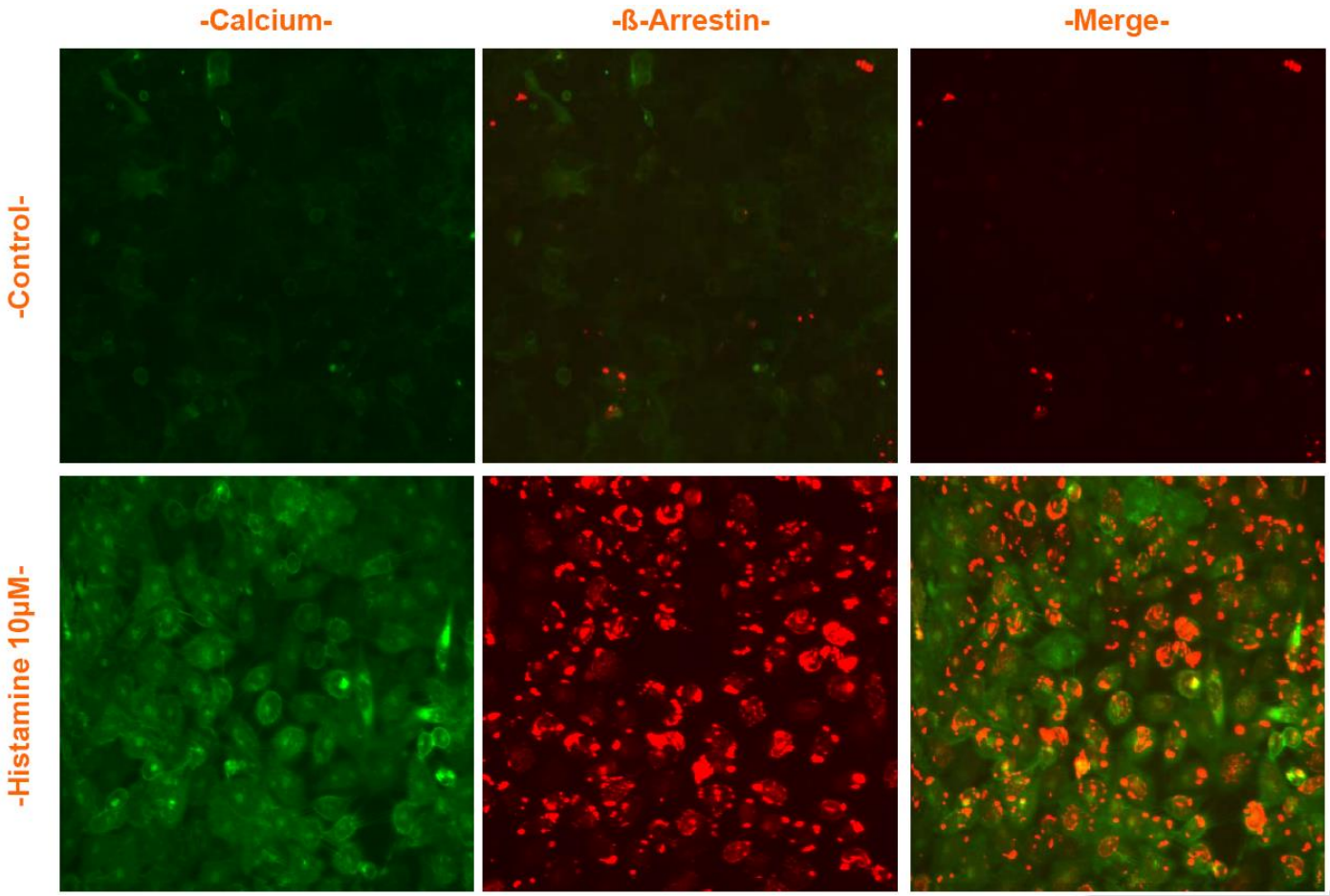
REF: P70735

MULTIPLEX CELL LINES – Calcium and ß-Arrestin

MPXNOMAD HISTAMINE RECEPTOR H1



MPXNomad-HRH1 (U2OS cell line)

Ec_{50} ß-Arrestin1 assay: $3.86x10^{-8}$ M

Ec_{50} calcium assay: $6.93x10^{-8}$ M

$Z'_{ß\text{-}Arrestin1}$: 0.83+/- 0.01

$Z'_{Calcium}$: 0.55+/- 0.01

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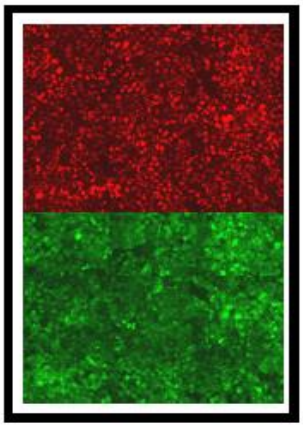

Product Name: HRH1 $_{MPX}$Nomad cell line
Reference: P70735
Recp. Official Full Name: Histamine Receptor H1
DNA Accession Number: AY136743
Host Cell: U2OS
Resistance: G418 + Puromycin + Hygromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of $_{MPX}$Nomad-HRH1 contains U2OS cells stably expressing red $_{ß\text{-}Arrestin}$Nomad and green $_{Ca2+}$Nomad biosensor and Histamine receptor H1(with no tag).

Innoprot´s $_{MPX}$Nomad-HRH1 cell line has been designed to assay compounds or analyze their capability to modulate Histamine receptor H1. When an agonist binds to HRH1 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 Histamine 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 Histamine receptor H1 (HRH1), were stimulated with 8 log dilution series ranging from 0 to 10 µM of Histamine during 24h (n=5). % Activity was calculated relative to positive (10µM).

Image analysis

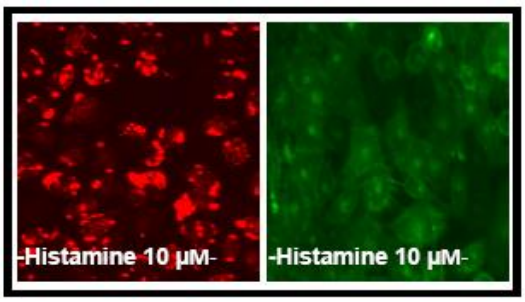

Fig1. ß-arrestin-Ca^{2+} $_{MPX}$Nomad biosensor stimulated with 10µM of Histamine.

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 ~ $6.93x10^{-7}$M validated with an average of Z´= 0.55+/-0.02 and for calcium assay the Ec50 was ~ $3.86x10^{-8}$M and Z´=0.83

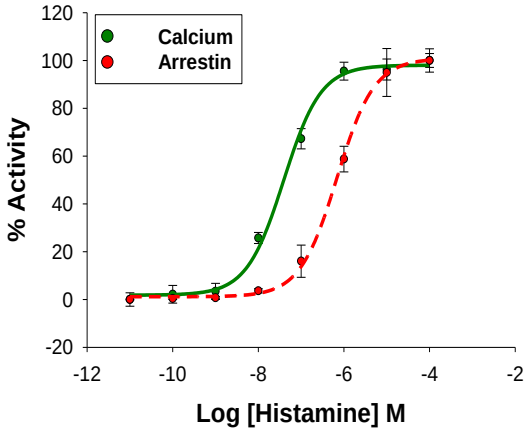

Fig2. Concentration response curve for Histamine in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-HRH1 cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

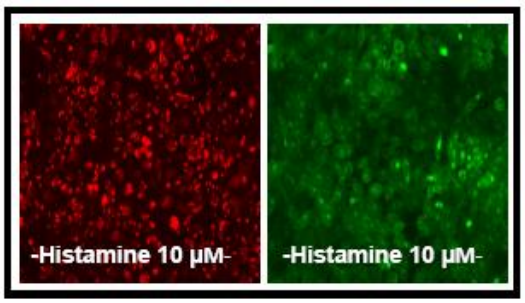

Fig3. ß-arrestin-Ca^{2+} $_{MPX}$Nomad biosensor stimulated with 10µM of Histamine.

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 ~ $1.03x10^{-6}$M validated with an average of Z´= 0.59+/-0.02 and for calcium assay the Ec50 was ~ $2.90x10^{-8}$M and Z´=0.87

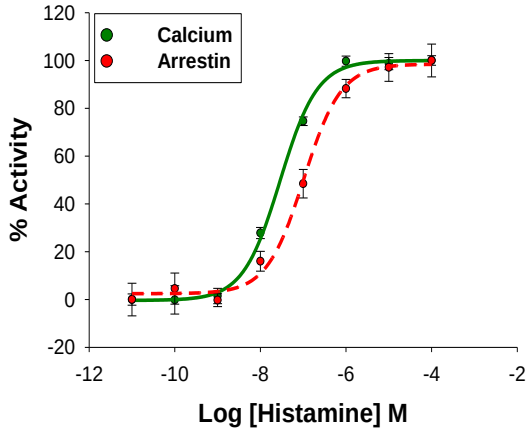

Fig4. Concentration response curve for Histamine in ß-arrestin-Ca^{2+} $_{MPX}$Nomad-HRH1 cell line analyzed using a microplate reader.

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