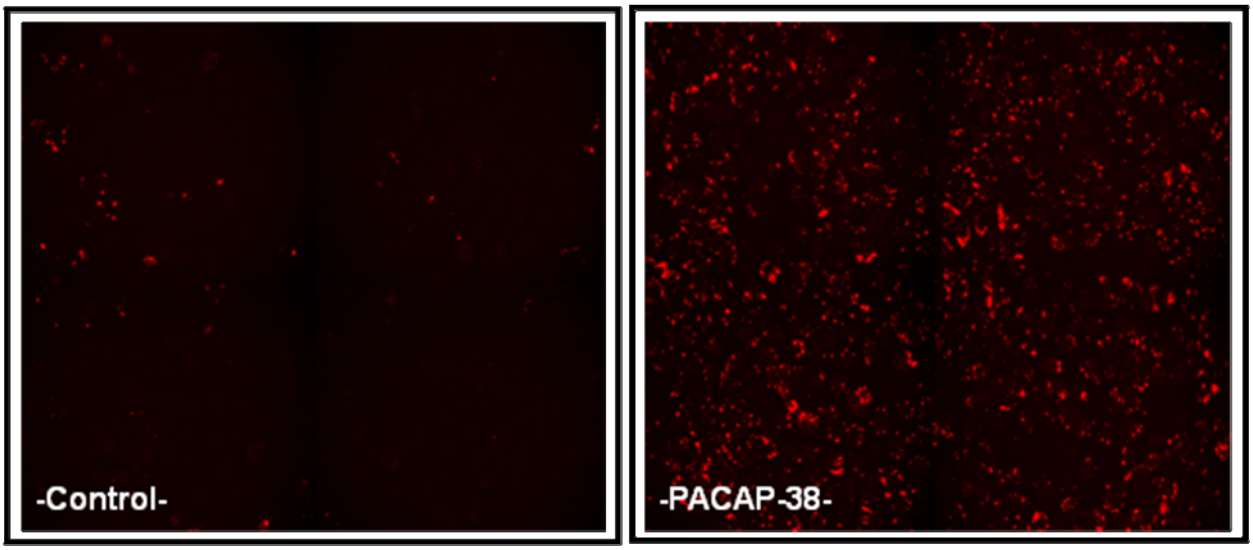
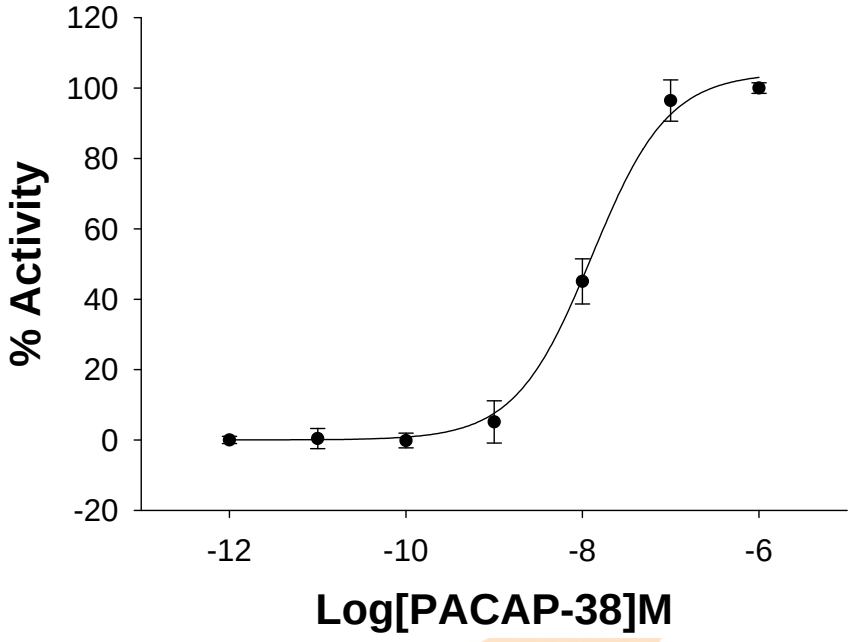
REF: P70569

cAMP NOMAD CELL LINES

-VASOACTIVE INTESTINAL PEPTIDE RECEPTOR 2 (VIPR2)-





Red $_{cAMP}$Nomad-VIPR2 (U2OS cell line)

Ec_{50} PACAP-38: $1.28x10^{-8}$ M

Z´: 0.93+/- 0.01

VAT No. ESB95481909

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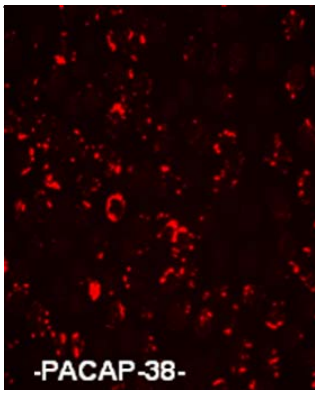


Product Name: VIPR2 cAMPNomad cell line

Reference: P70569

Recp. Official Full Name: Vasoactive intestinal peptide receptor 2

DNA Accession Number: NM_003382.4

Host Cell: U2OS

Resistance: G418 + Puromycin

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of red cAMPNomad-VIPR2 contains U2OS cells stably expressing red cAMPNomad biosensor and Vasoactive intestinal peptide receptor 2 (with no tag).

Innoprot´s red cAMPNomad-VIPR2 cell line has been designed to assay compounds or analyze their capability to modulate Vasoactive intestinal peptide receptor 2. When an agonist binds to VIPR2 a G protein is activated, which in turn, triggers a cellular response mediated by cAMP.

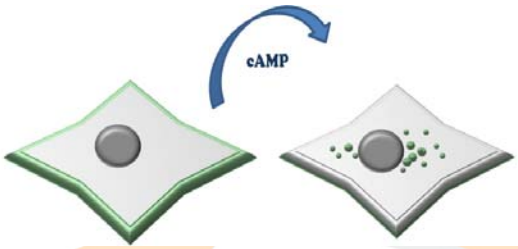
This cell line has been validated measuring cAMP increase in the cytosol analyzing red cAMPNomad biosensor distribution within the cell. This cell line allows the image analysis of the stimuli induced by the compounds.

This highly reproducible assay has been validated using human PACAP-38 as agonist in a High Content Analysis (HCA) and a High Throughput Analysis (HTA).

About Red cAMPNomad Biosensor

Red cAMPNomad Biosensor is a fluorescent polypeptide that in the presence or absence of cAMP changes its localization within the cell.

Before cAMP production stimulation, 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 red cAMPNomad Biosensor promoting its cellular relocation in the vesicular trafficking of the cells.



In a cell line co-expressing red cAMPNomad Biosensor and a GPCR of interest, the activity can be easily quantified on living cells by image analysis of fluorescence granularity or fluorescence intensity analysis.

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cAMP Assay

cAMPNomad U2OS cells, stably expressing Vasoactive intestinal peptide receptor 2 (VIPR2), were stimulated with 8 log dilution series ranging from 0 to 1 µM of PACAP-38 during 24h (n=5). % Activity was calculated relative to positive (1 µM).

Image analysis

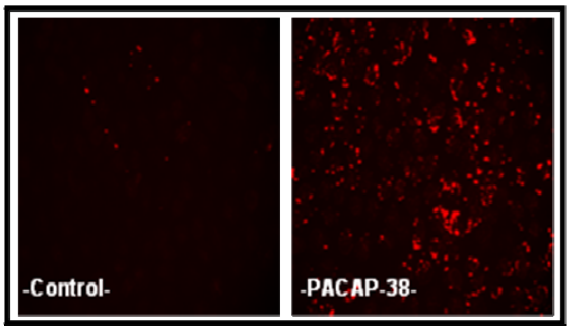

Fig1. Red cAMPNomad biosensor negative control and PACAP-38 stimulation.

Activation and biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The EC50 for PACAP-38 was ~ $1.28x10^{-8}$M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.93+/-0.01.

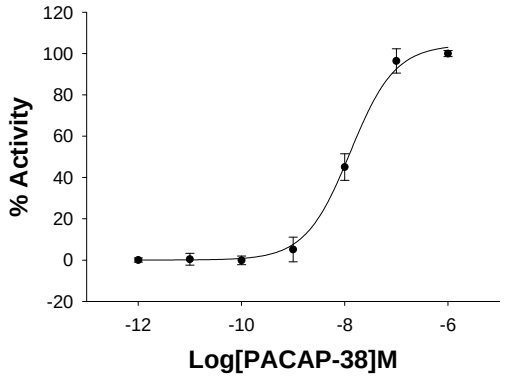

Fig2. Concentration response curve for PACAP-38 in Red cAMPNomad-VIPR2 cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

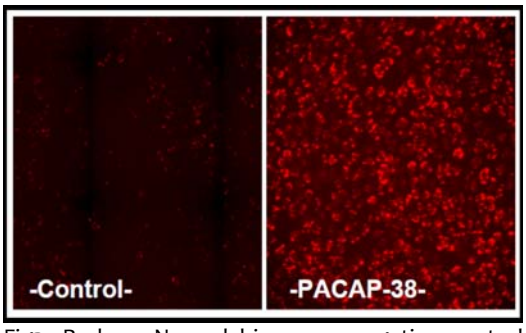

Fig3. Red cAMPNomad biosensor negative control and PACAP-38 stimulation.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for PACAP-38 was ~ $1.25x10^{-8}$M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.74+/-0.01.

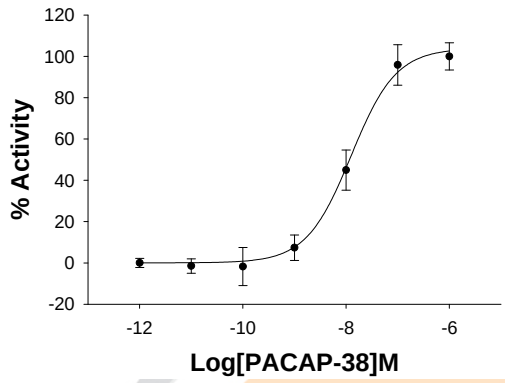

Fig4. Concentration response curve for PACAP-38 in Red cAMPNomad-VIPR2 cell line analyzed using a microplate reader.

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