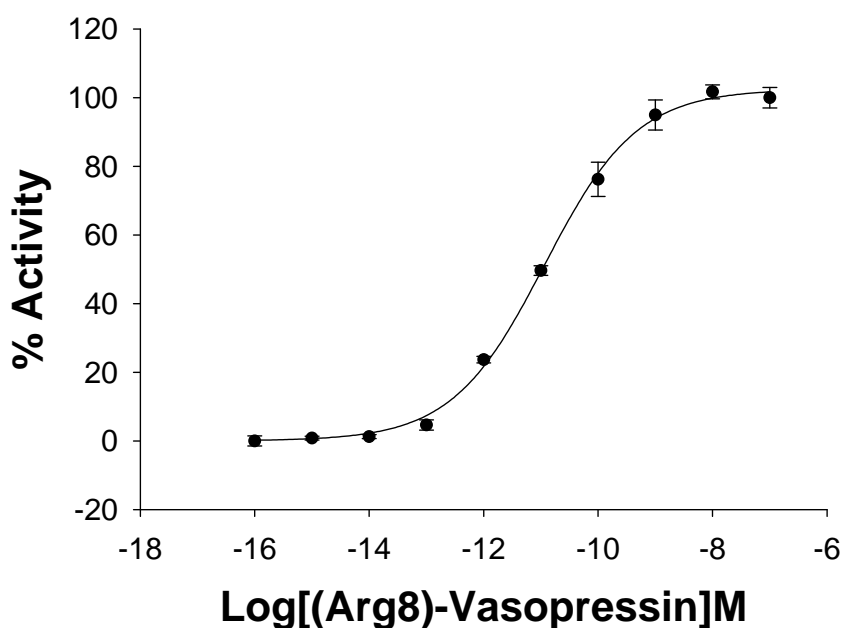
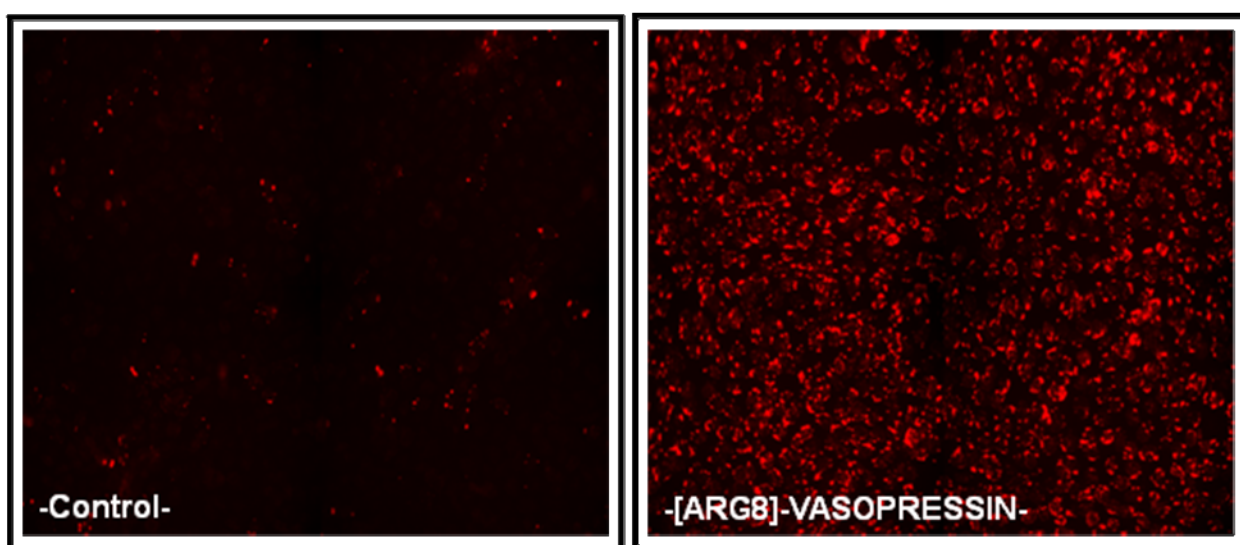


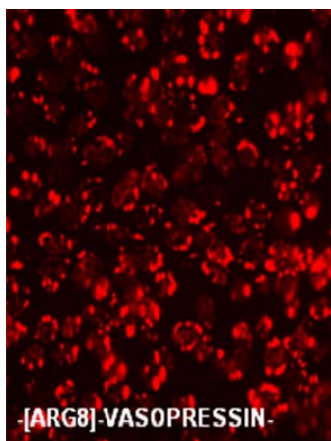
cAMP⁺NOMAD CELL LINES
ARGININE VASOPRESSIN RECEPTOR 2 (AVPR2)



Red ^{cAMP}Nomad-AVPR2 (U2OS cell line)

EC₅₀ [Arg8]-Vasopressin: 1.16x10⁻¹¹ M

Z': 0.87+/- 0.01



Product Name: AVPR2 _{cAMP}Nomad cell line

Reference: P70517

Official Full Name: Arginine Vasopressin receptor 2

DNA Accession Number: NM_000706.3

Host Cell: U2OS

Resistance: G418 + Puromycin

Quantity: > 3 x 10⁶ cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of red _{cAMP}Nomad-AVPR2 contains U2OS cells stably expressing red _{cAMP}Nomad biosensor and Vasopressin receptor 2 (with no tag).

Innoprot's red _{cAMP}Nomad-AVPR2 cell line has been designed to assay compounds or analyze their capability to modulate Vasopressin receptor 2. When an agonist binds to AVPR2 a G protein is activated, which in turn, triggers a cellular response mediated by cAMP.

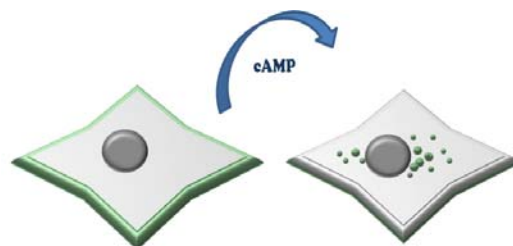
This cell line has been validated measuring cAMP increase in the cytosol analyzing red _{cAMP}Nomad biosensor distribution within the cell. This cell line allows the image analysis of the stimuli induced by the compounds.

This highly reproducible assay has been validated using [Arg8]-Vasopressin as agonist in a High Content Analysis (HCA) and a High Throughput Analysis (HTA).

About Red _{cAMP}Nomad Biosensor

Red _{cAMP}Nomad 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 _{cAMP}Nomad Biosensor promoting its cellular relocation in the vesicular trafficking of the cells.



In a cell line co-expressing red _{cAMP}Nomad 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.



cAMP Assay

$cAMP$ Nomad U2OS cells, stably expressing Vasopressin receptor 2 (AVPR2), were stimulated with 10 log dilution series ranging from 0 to 100 nM of [Arg8]-Vasopressin during 24h (n=4). % Activity was calculated relative to positive (100 nM).

Image analysis

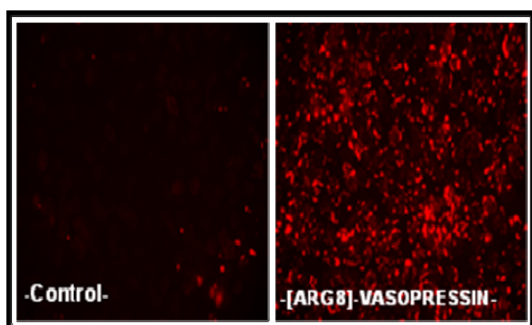


Fig1. Red $cAMP$ Nomad biosensor negative control and [Arg8]-Vasopressin stimulation.

Activation and biosensor change of localization processes were detected and analyzed using “BD Pathway 855” High-Content Bioimager from BD Biosciences. The EC_{50} for [Arg8]-Vasopressin was $\sim 1.16 \times 10^{-11} M$ after a treatment of 24 h with the agonist. The assay was validated with an average of $Z' = 0.87 \pm 0.01$.

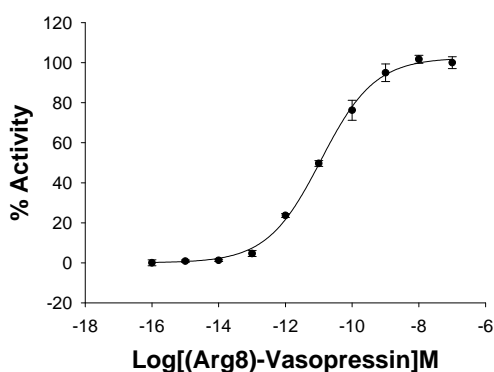


Fig2. Concentration response curve for [Arg8]-Vasopressin in Red $cAMP$ Nomad-AVPR2 cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

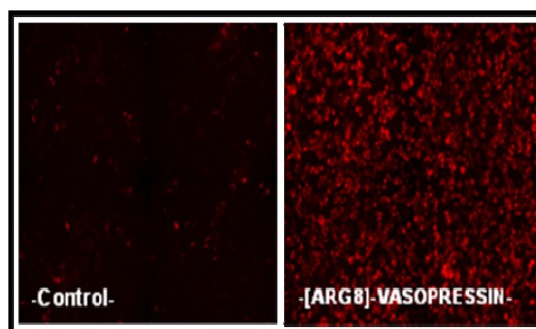


Fig3. Red $cAMP$ Nomad biosensor negative control and [Arg8]-Vasopressin stimulation.

The increase in the fluorescence was detected and analyzed using “Synergy 2” microplate reader from Biotek. The EC_{50} for [Arg8]-Vasopressin was $\sim 2.39 \times 10^{-11} M$ after a treatment of 24 h with the agonist. The assay was validated with an average of $Z' = 0.92 \pm 0.01$.

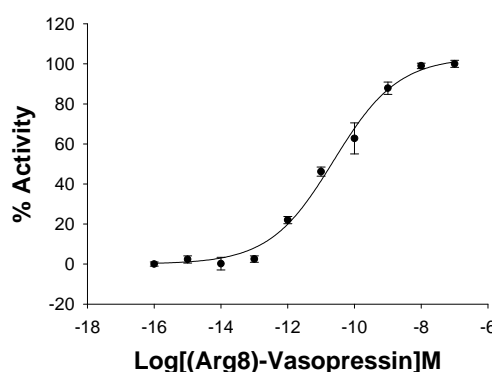


Fig4. Concentration response curve for [Arg8]-Vasopressin in Red $cAMP$ Nomad-AVPR2 cell line analyzed using a microplate reader.