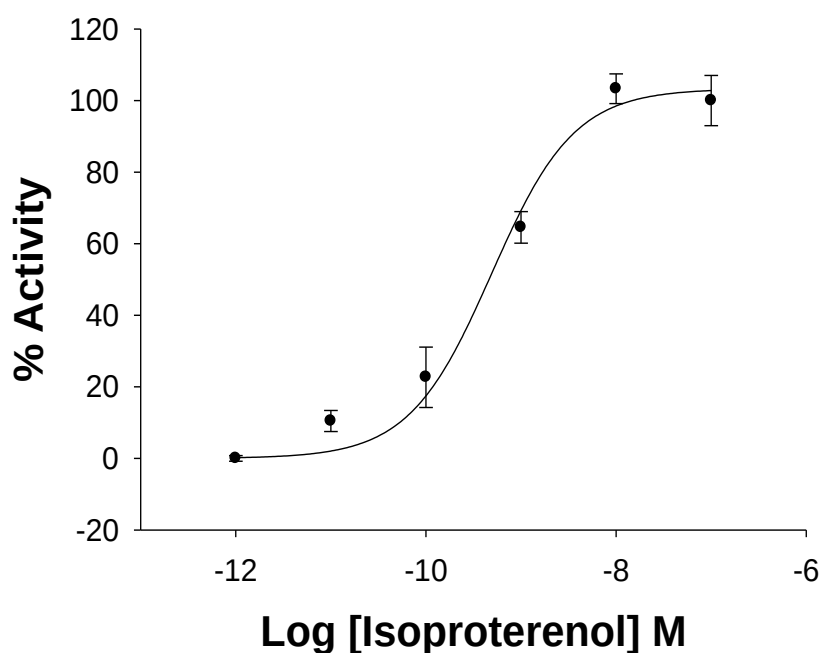
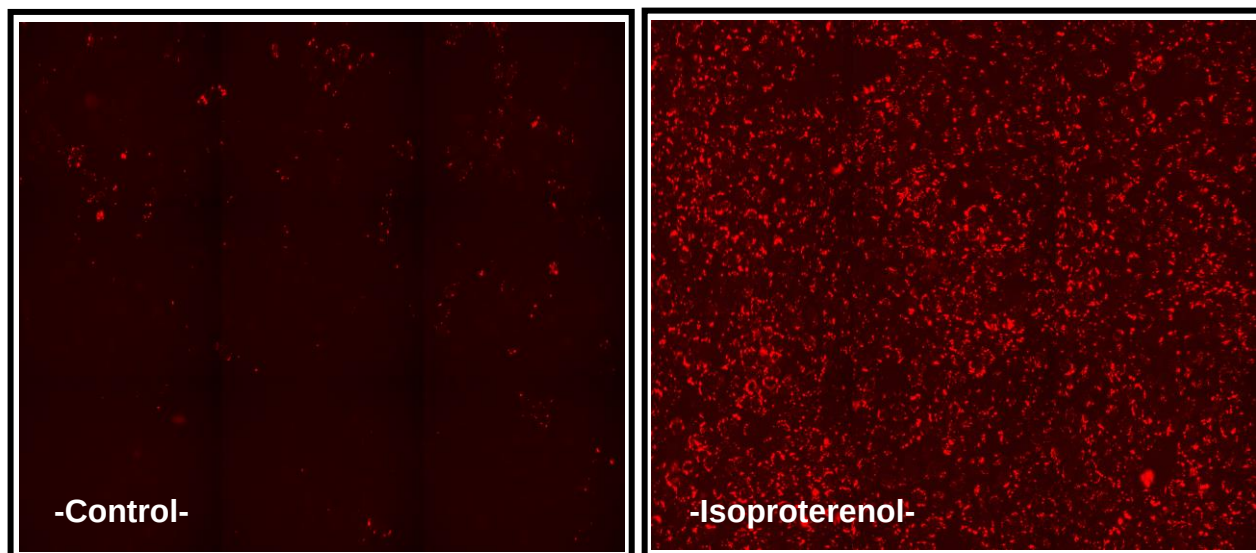


cAMP NOMAD-FP650 CELL LINES

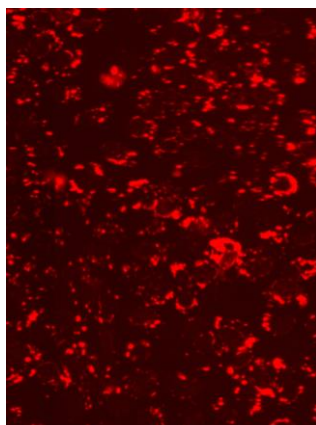
-ADRENOCEPTOR BETA 2 (ADRB2)-



Red _{cAMP}Nomad-ADRB₂ (U2OS cell line)

EC₅₀ Isoproterenol: 4.91×10^{-10} M

Z': 0.77 +/- 0.01



Product Name: ADRβ2 _{cAMP}Nomad cell line

Reference: P70205

Recp. Official Full Name: Adrenoreceptor beta 2

DNA Accession Number:

Host Cell: U2OS

Resistance: G418 + Puromycin

Quantity: > 3 x 10⁶ cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of _{cAMP}Nomad ADRβ2 contains U2OS cells stably expressing _{cAMP}Nomad-FP650 biosensor and adrenoreceptor beta 2 (with no tag).

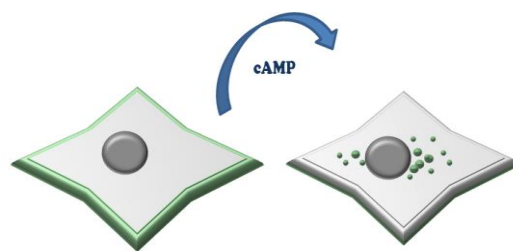
Innoprot _{cAMP}Nomad ADRβ2 cell line has been designed to assay compounds or analyze their capability to modulate adrenoreceptor beta 2. When an agonist binds to ADRβ2 a G protein is activated, which in turn, triggers a cellular response mediated by cAMP. This cell line has been validated measuring cAMP increase in the cytosol analyzing _{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 Isoproterenol 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

cAMPNomad U2OS cells, stably expressing adrenoreceptor beta 2 (ADRB₂), were stimulated with 10 log dilution series ranging from 0 to 100 μ M of Isoproterenol during 24h (n=5). % Activity was calculated relative to positive (100 μ M).

Image analysis

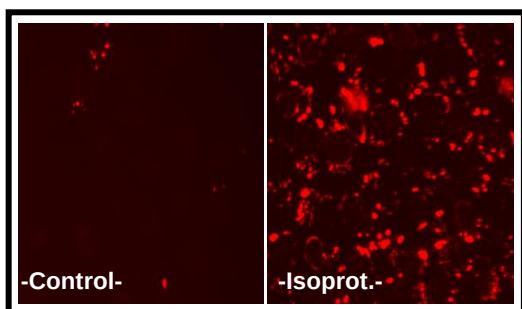


Fig1. Red cAMPNomad biosensor negative control and Isoproterenol stimulation.

Activation and biosensor change of localization processes were detected and analyzed using “BD Pathway 855” High-Content Bioimager from BD Biosciences. The EC₅₀ for Isoproterenol was $\sim 4.91 \times 10^{-10}$ M after a treatment of 24 h with the agonist. The assay was validated with an average of $Z' = 0.77 \pm 0.02$.

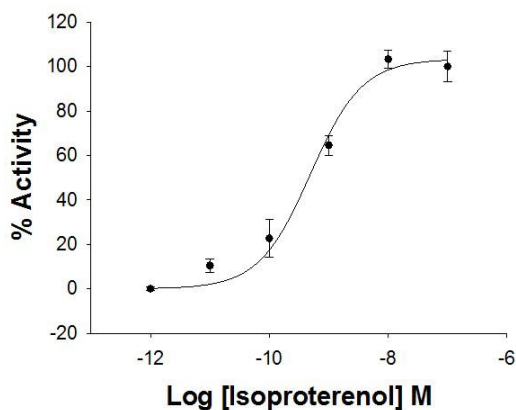


Fig2. Concentration response curve for Isoproterenol in Red cAMPNomad-ADRB₂ cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

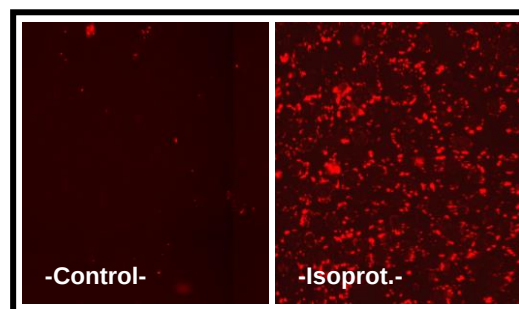


Fig3. Red cAMPNomad biosensor negative control and Isoproterenol stimulation.

The increase in the fluorescence was detected and analyzed using “Synergy 2” microplate reader from Biotek. The EC₅₀ for Isoproterenol was $\sim 3.72 \times 10^{-10}$ M after a treatment of 24 h with the agonist. The assay was validated with an average of $Z' = 0.65 \pm 0.02$.

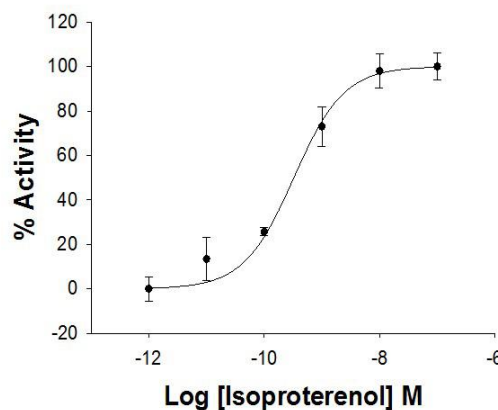


Fig4. Concentration response curve for Isoproterenol in Red cAMPNomad-ADRB₂ cell line analyzed using a microplate reader.