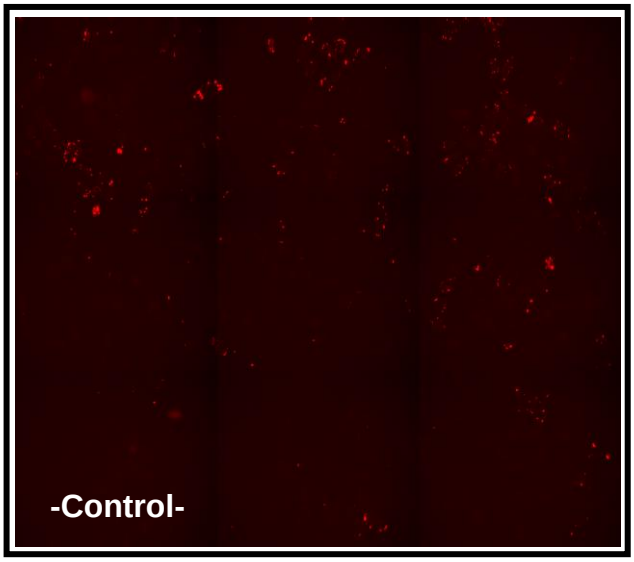
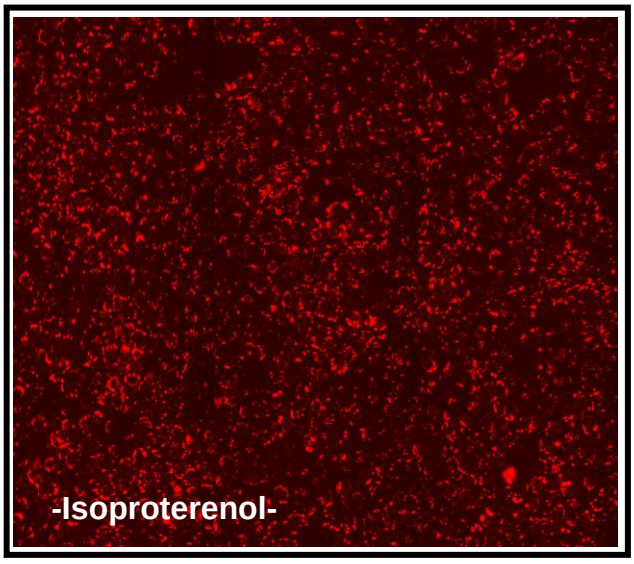
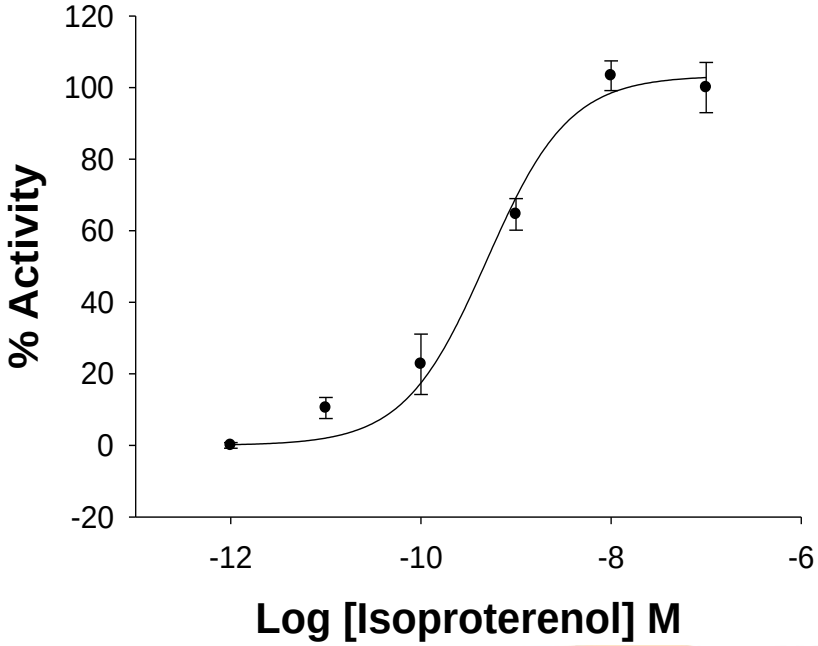
REF: P70205

cAMP NOMAD-FP650 CELL LINES

-ADRENOCEPTOR BETA 2 (ADRB2)-







Red $_{cAMP}$Nomad-ADRß2 (U2OS cell line)

Ec_{50} Isoproterenol: $4.91x10^{-10}$ M

Z´: 0.77+/- 0.01

VAT No. ESB95481909

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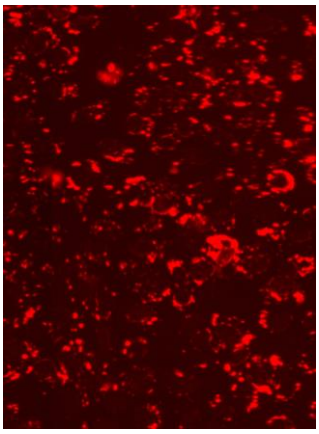


Product Name: ADRß2 cAMPNomad cell line
Reference: P70205
Recp. Official Full Name: Adrenoreceptor beta 2
DNA Accession Number:
Host Cell: U2OS
Resistance: G418 + Puromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of cAMPNomad ADRß2 contains U2OS cells stably expressing cAMPNomad-FP650 biosensor and adrenoreceptor beta 2 (with no tag).

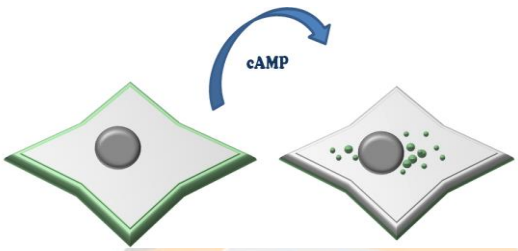
Innoprot cAMPNomad ADRß2 cell line has been designed to assay compounds or analyze their capability to modulate adrenoreceptor beta 2. When an agonist binds to ADRß2 a G protein is activated, which in turn, triggers a cellular response mediated by cAMP. This cell line has been validated measuring cAMP increase in the cytosol analyzing 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 Isoproterenol 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 adrenoreceptor beta 2 (ADRß2), were stimulated with 10 log dilution series ranging from 0 to 100 µM of Isoproterenol during 24h (n=5). % Activity was calculated relative to positive (100µM).

Image analysis

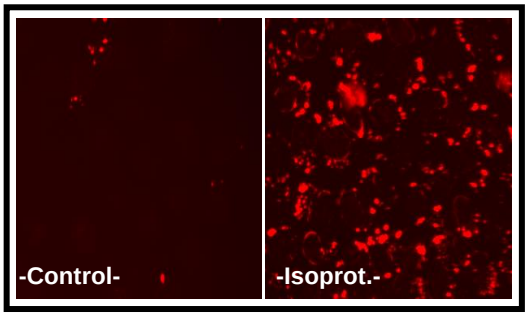

Fig1. Red cAMPNomad biosensor negative control and Isoproterenol stimulation.

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 ~ $4.91x10^{-10}$M after a treatment of 24 h with the agonist.The assay was validated with an average of Z´= 0.77+/-0.02.

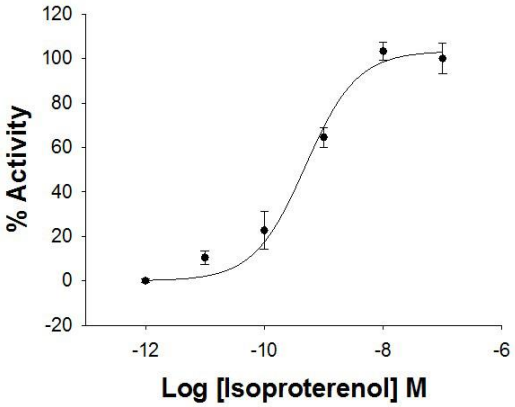

Fig2. Concentration response curve for Isoproterenol in Red cAMPNomad-ADRß2 cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

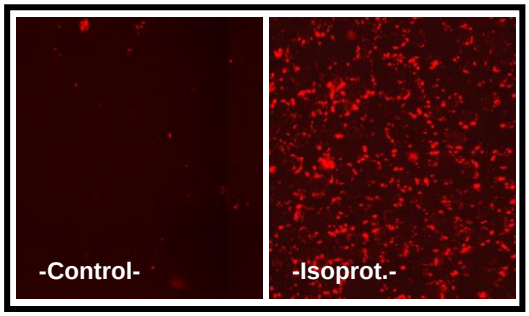

Fig3. Red cAMPNomad biosensor negative control and Isoproterenol stimulation.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for Isoproterenol was ~ $3.72x10^{-10}$M after a treatment of 24 h with the agonist.The assay was validated with an average of Z´= 0.65+/-0.02.

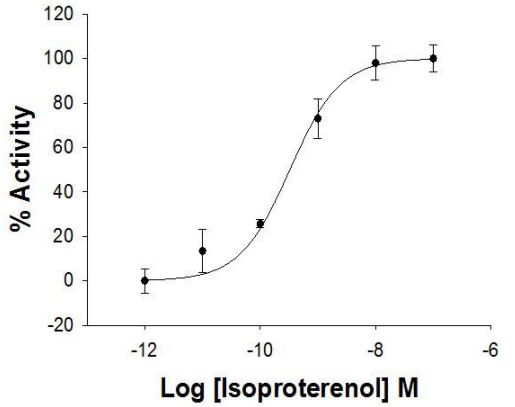

Fig4. Concentration response curve for Isoproterenol in Red cAMPNomad-ADRß2 cell line analyzed using a microplate reader.

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