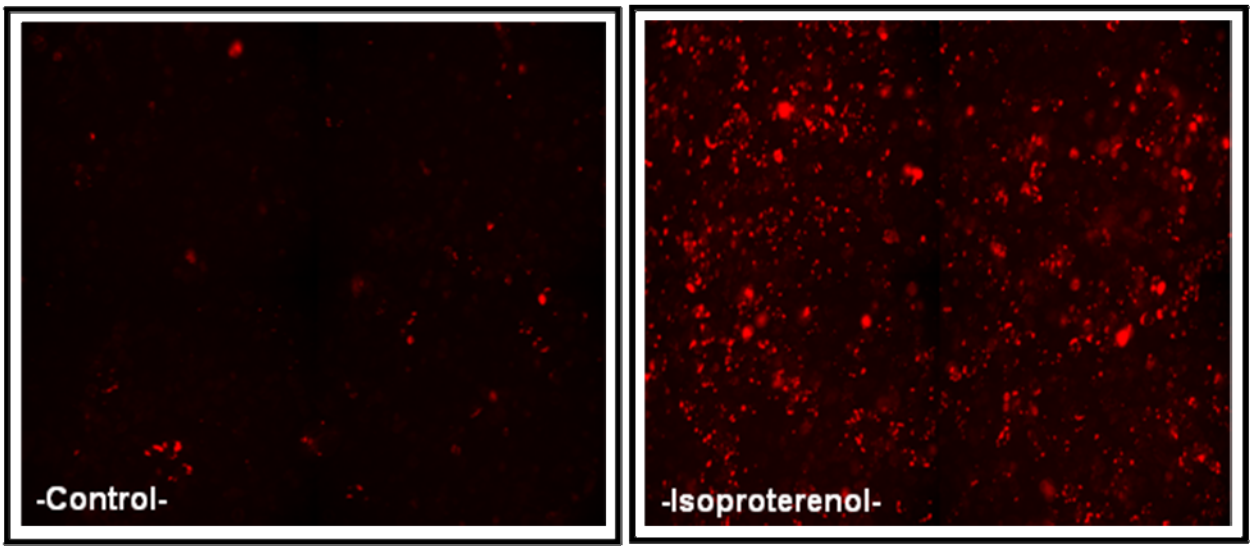
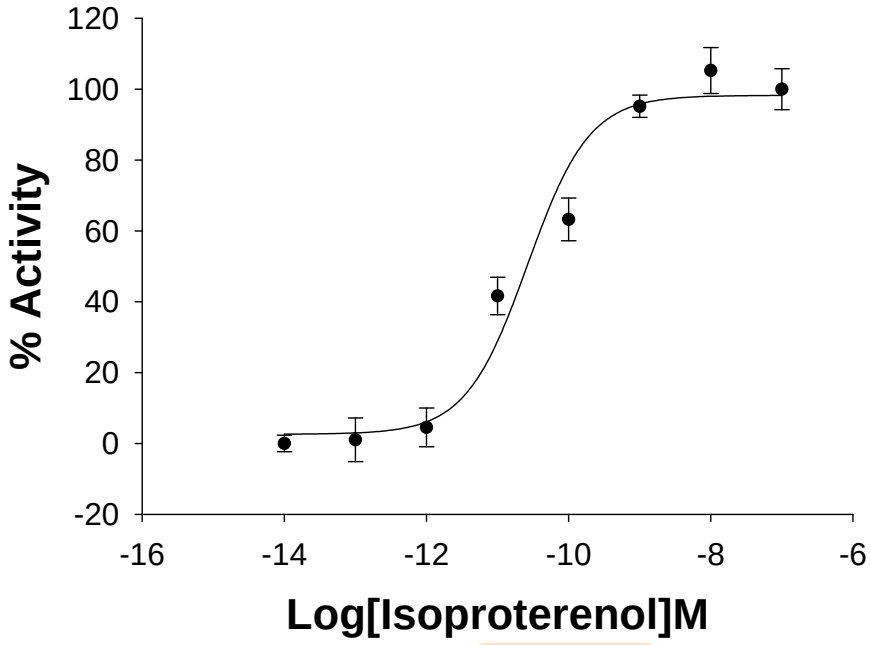
REF: P70506

cAMP NOMAD-FP650 CELL LINES

ADRENOCEPTOR BETA 3 (ADRB3)





Red cAMPNomad-ADRß3 (U2OS cell line)

Ec50 Isoproterenol: 2.64 x 10^{-11} M

Z´: 0.70 +/- 0.01

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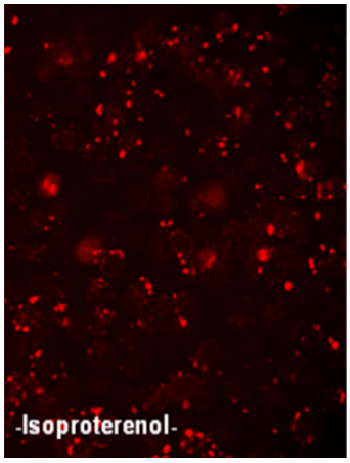


Product Name: ADRB3 $_{cAMP}$Nomad cell line
Reference: P70506
Recp. Official Full Name: Adrenoceptor Beta 3
DNA Accession Number: AY487247
Host Cells: U2OS Cell Line
Resistance: G418 + Puromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of red $_{cAMP}$Nomad-ADRß3 contains U2OS cells stably expressing red $_{cAMP}$Nomad biosensor and Adrenoceptor Beta 3 (with no tag).

Innoprot´s $_{cAMP}$Nomad-ADRß3 cell line has been designed to assay compounds or analyze their capability to modulate Adrenoceptor Beta 3. When an agonist binds to ADRß3 a G protein is activated, which in turn, triggers a cellular response mediated by cAMP.

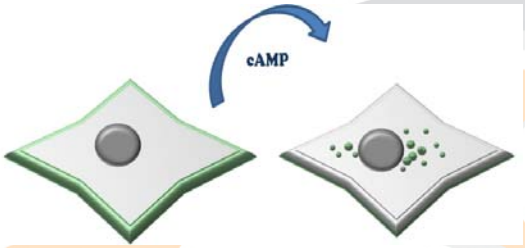
This cell line has been validated measuring cAMP increase in the cytosol analyzing both red fluorescence increase and biosensor distribution within the cell.

This highly reproducible assay has been validated using Isoproterenol as agonist in a High Throughput Screening (HTS) and High Content Analysis (HCA).

About Red $_{cAMP}$Nomad Biosensor

Red $_{cAMP}$Nomad Biosensor is a fluorescent polypeptide that in the presence or absence of cAMP changes its fluorescence intensity and 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.

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cAMP Assay

cAMPNomad U2OS cells, stably expressing Adrenoceptor Beta 3 (ADRß3), were stimulated with 8 log dilution series ranging from 0 to 100 nM of Isoproterenol during 24h (n=4). % Activity was calculated relative to positive (100 nM).

Image analysis

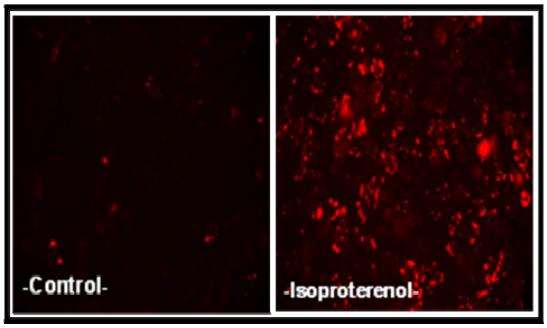

Fig1. Red cAMPNomad biosensor negative control and Isoproterenol stimulation.

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 ~ $3.04x10^{-11}$M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.69+/-0.01.

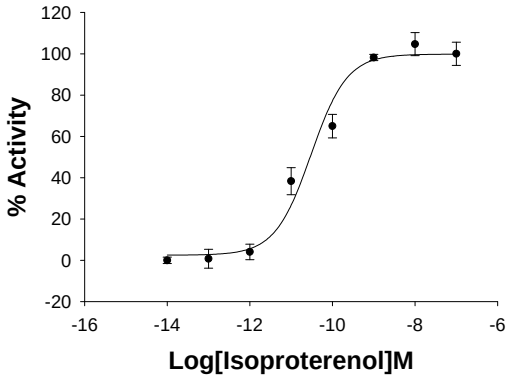

Fig2. Concentration response curve for Isoproterenol in Red cAMPNomad-ADRß3 cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

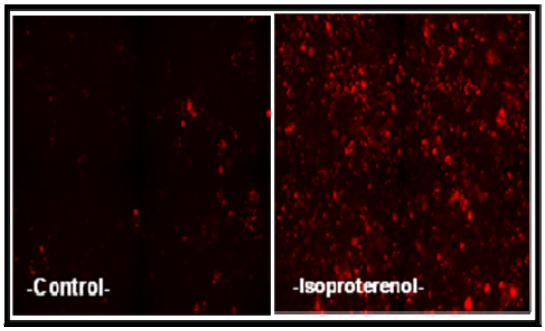

Fig3. Red cAMPNomad biosensor negative control and Isoproterenol stimulation.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for Isoproterenol was ~ $2.64x10^{-11}$M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.70+/-0.01.

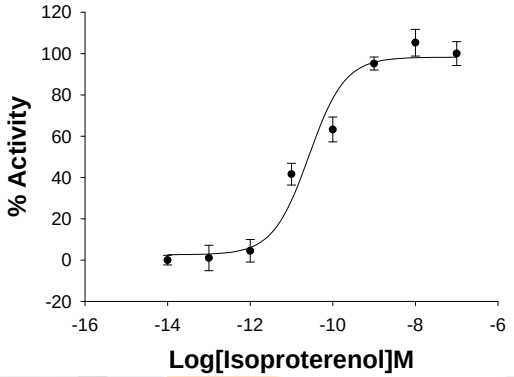

Fig4. Concentration response curve for Isoproterenol in Red cAMPNomad-ADRß3 cell line analyzed using a microplate reader.

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