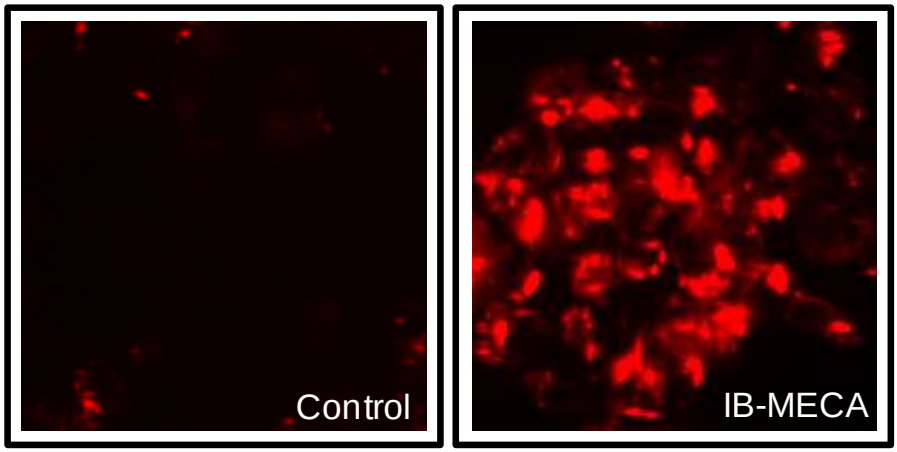
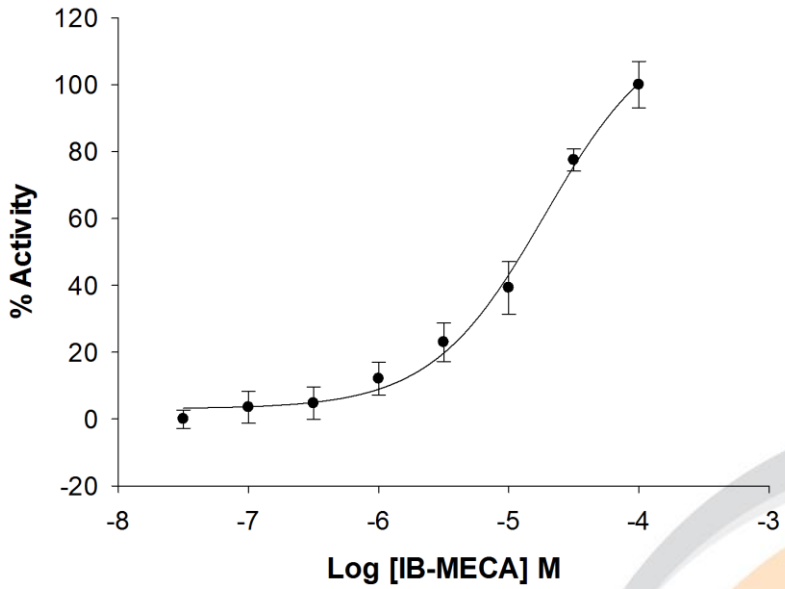
REF: P70524

cAMP NOMAD-FP650 cell lines

-ADENOSINE A3 RECEPTOR (ADORA3)-





Red $_{cAMP}$Nomad-ADORA3 (HEK293 cell line)

EC_{50} IB-MECA: $1{,}88 \times 10^{-5}$ M

Z´: 0.71+/- 0.01

VAT No. ESB95481909

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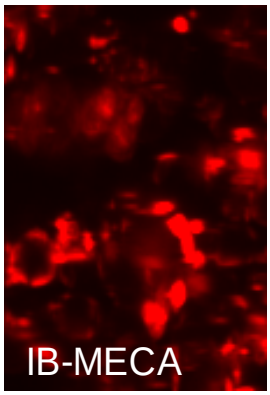

Product Name: ADORA3 cAMPNomad cell line
Reference: P70524
Recp. Official Full Name: Adenosine A3 receptor
DNA Accession Number: NM_000677
Host Cell: HEK293
Resistance: G418 + Hygromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of cAMPNomad ADORA3 contains HEK293 cells stably expressing cAMPNomad-FP650 biosensor and adenosine A3 receptor (with no tag).

Innoprot cAMPNomad ADORA3 cell line has been designed to assay compounds or analyze their capability to modulate Adenosine A3 receptor. When an agonist binds to ADORA3 a G protein is activated, which in turn, triggers a cellular response mediated by cAMP. This cell line has been validated measuring cAMP decrease 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 IB-MECA as agonist in 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/decrease 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

Red cAMPNomad HEK293 cells, stably expressing adenosine A3 receptor (ADORA3), were stimulated with 8 log dilution series ranging from 0 to 100 µM of IB-MECA during 24h (n=5). % Activity was calculated relative to positive (100 µM).

Fluorescence intensity analysis

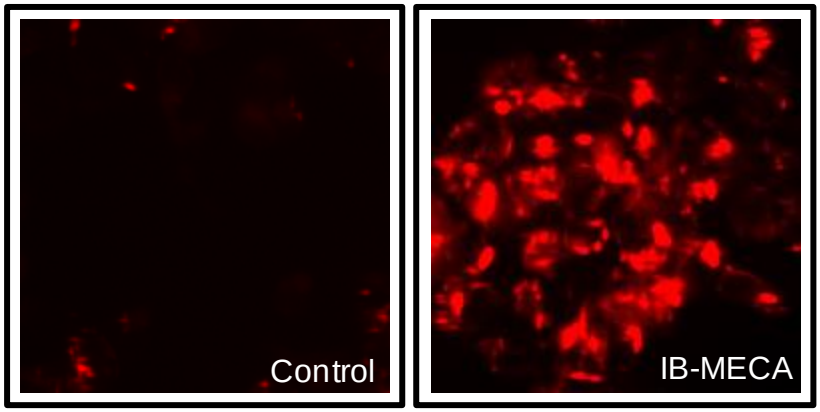

Fig1. Red cAMPNomad biosensor negative control and IB-MECA stimulation.

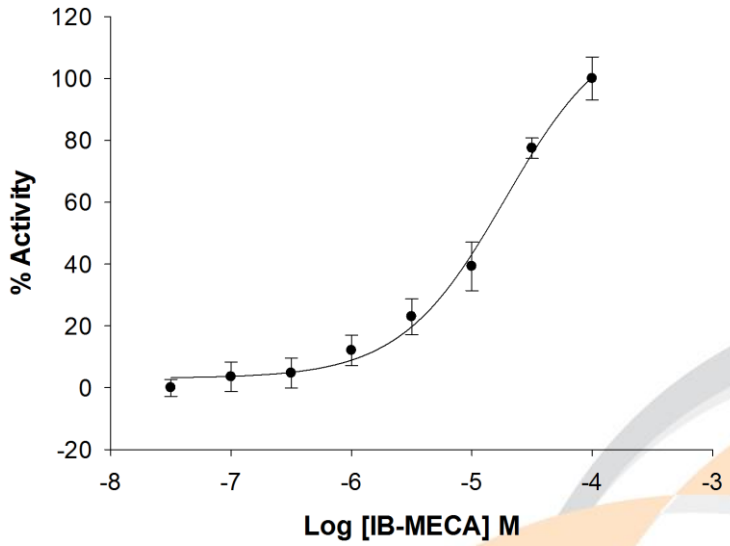

Fig 2. Concentration-response curve for adenosine A3 receptor in Red cAMPNomad-ADORA3 cell line analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for IB-MECA was $1{,}88 \times 10^{-5}$M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.71 +/- 0.01.

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