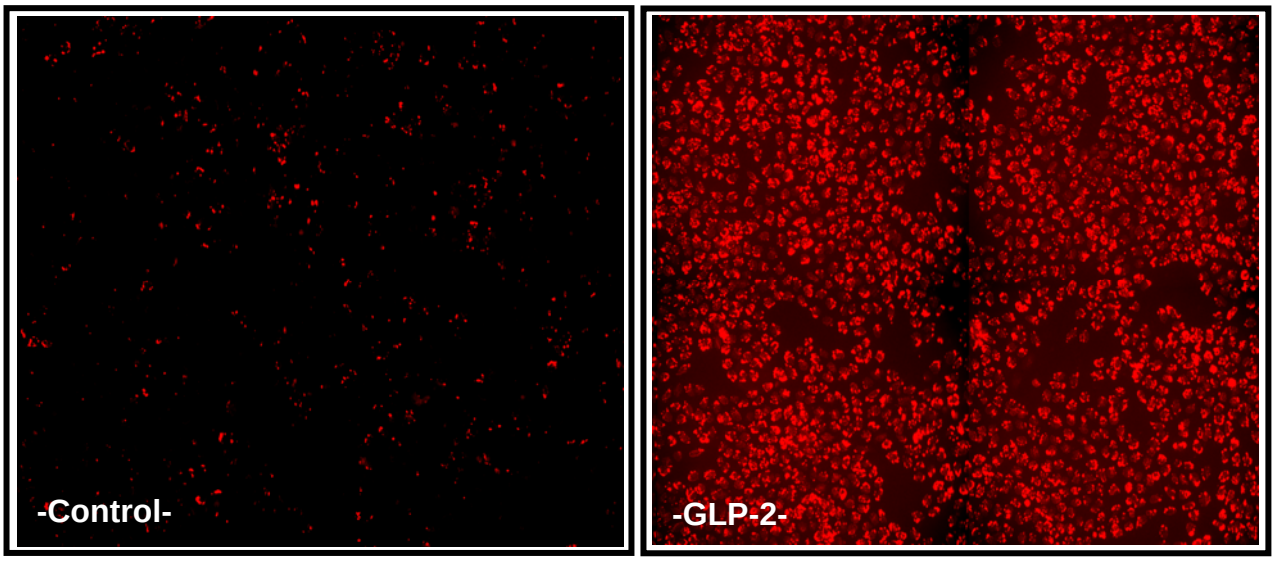
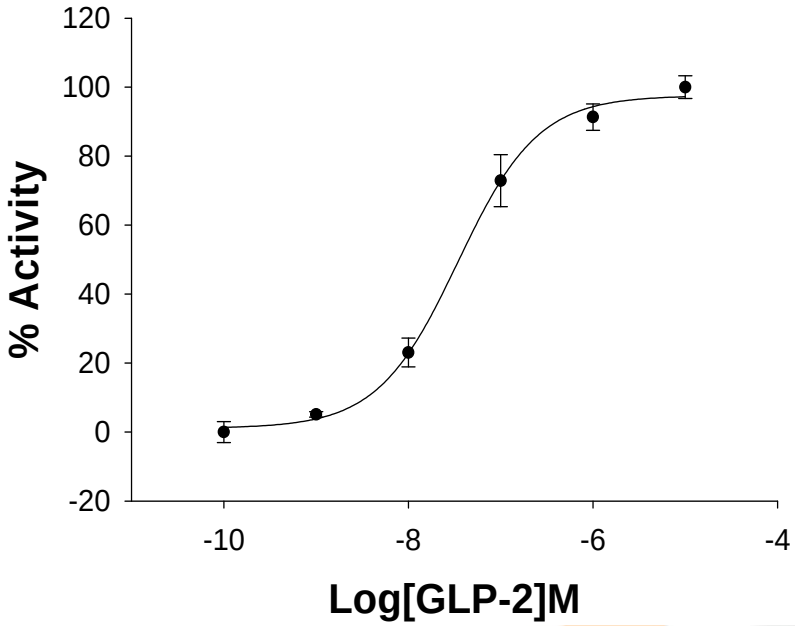
REF: P70514

cAMP NOMAD-FP650 CELL LINES

-GLUCAGON-LIKE PEPTIDE 2 RECEPTOR (GLP2R)-





Red $_{cAMP}$Nomad-GLP2R (U2OS cell line)

Ec_{50} GLP-2: $3.44x10^{-8}$ M

Z´: 0.81+/- 0.01

VAT No. ESB95481909

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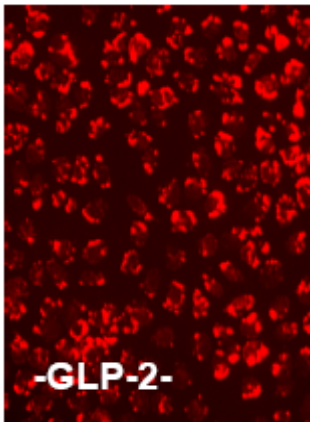


Product Name: GLP2R $_{cAMP}$Nomad cell line
Reference: P70514
Recp. Official Full Name: Glucagon-like peptide 2 receptor
DNA Accession Number: BC096261
Host Cell: U2OS
Resistance: G418 + Puromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of red $_{cAMP}$Nomad-GLP2R contains U2OS cells stably expressing red $_{cAMP}$Nomad biosensor and Glucagon-like peptide 2 receptor (with no tag).

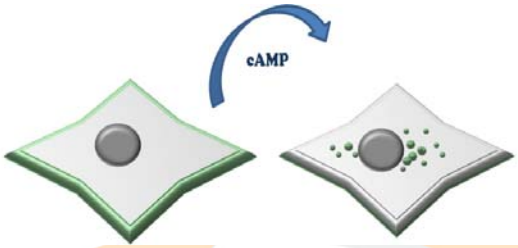
Innoprot´s red $_{cAMP}$Nomad-GLP2R cell line has been designed to assay compounds or analyze their capability to modulate Glucagon-like peptide 2 receptor. When an agonist binds to GLP2R a G protein is activated, which in turn, triggers a cellular response mediated by cAMP. This cell line has been validated 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 human GLP-2 as agonist in a High Content Analysis (HCA) and a High Throughput Screening (HTS).

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.

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cAMP Assay

cAMPNomad U2OS cells, stably expressing Glucagon-like peptide 2 receptor (GLP2R), were stimulated with 6 log dilution series ranging from 0 to 10 µM of GLP-2 during 24h (n=5). % Activity was calculated relative to positive (10µM).

Image analysis

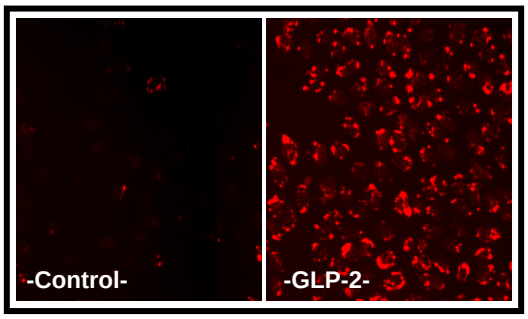

Fig1. Red cAMPNomad biosensor negative control and GLP-2 stimulation.

Activation and biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The EC50 for GLP-2 was ~ $3.44x10^{-8}$M after a treatment of 24 h with the agonist.The assay was validated with an average of Z´= 0.81+/-0.02.

Fluorescence intensity analysis

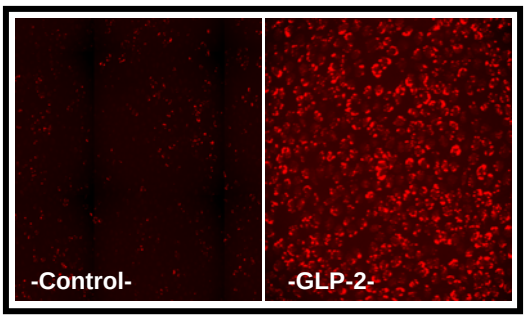

Fig2. Red cAMPNomad biosensor negative control and GLP-2 stimulation.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for GLP-2 was ~ $5.928x10^{-8}$M after a treatment of 24 h with the agonist.The assay was validated with an average of Z´= 0.72+/-0.01.

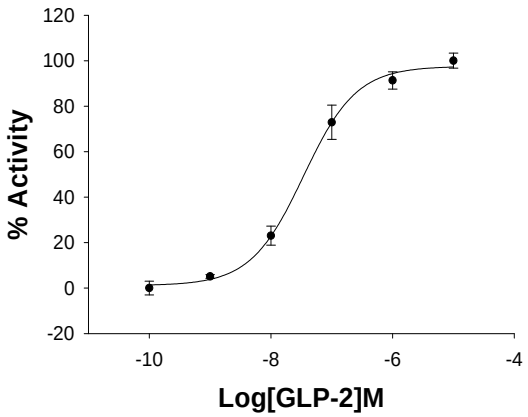

Fig3. Concentration response curve for GLP-2 in Red cAMPNomad-GLP2R cell line analyzed using a high-content bioimager.

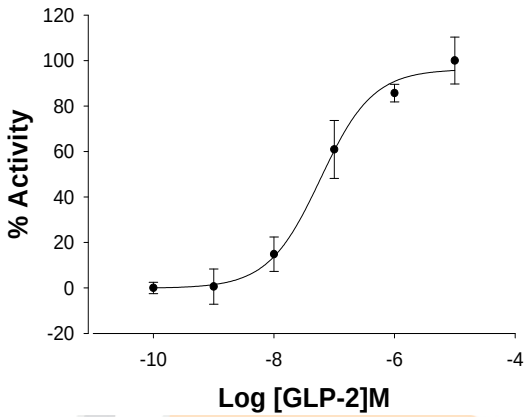

Fig4. Concentration response curve for GLP-2 in Red cAMPNomad-GLP2R cell line analyzed using a microplate reader.

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