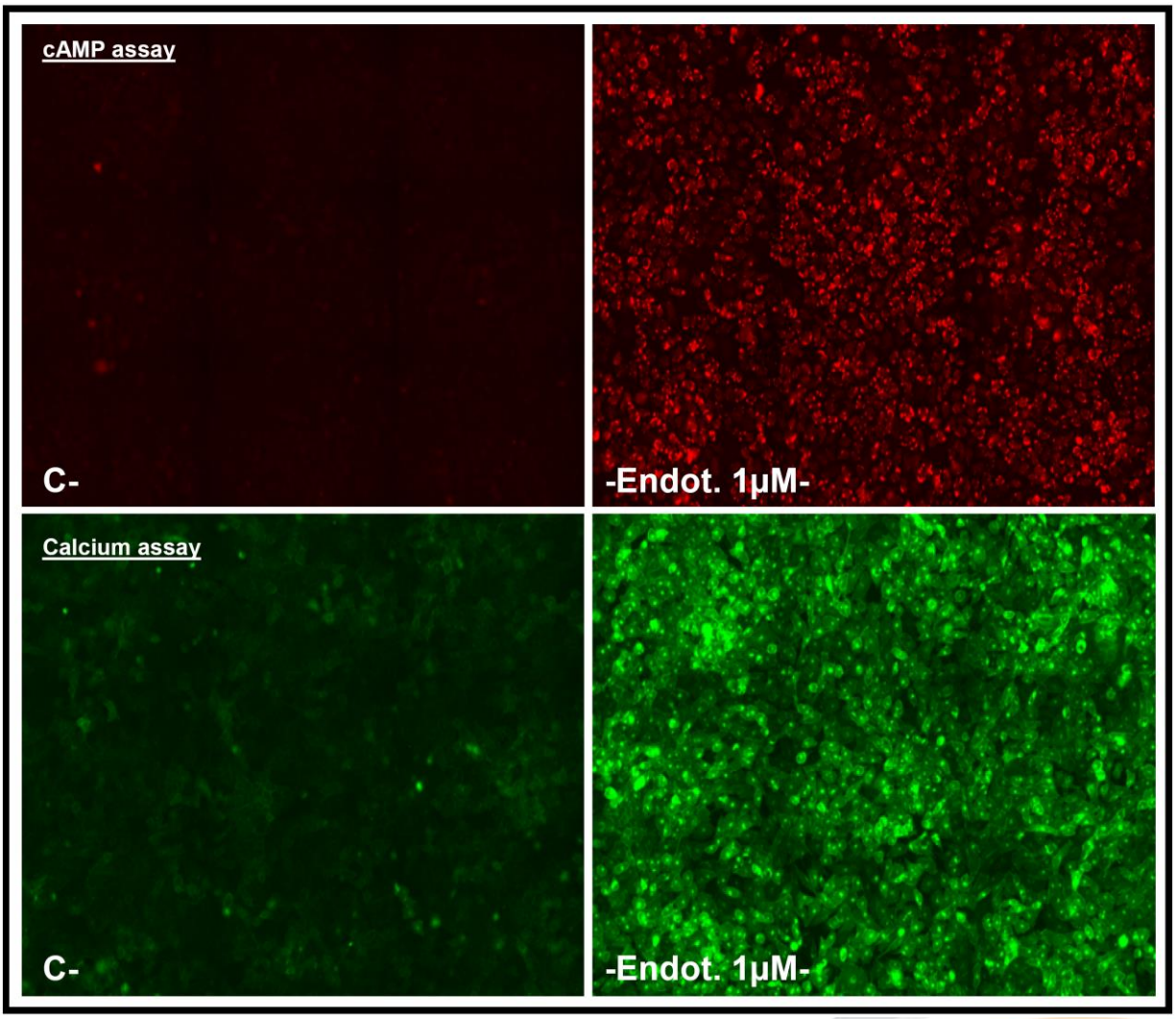
REF: P70752

MULTIPLEXED CELL LINES – cAMP & Calcium NOMAD

-ENDOTHELIN RECEPTOR TYPE B (ENDRB) NOMAD CELL LINE-



Multiplexed Nomad ENDRB (U2OS cell line)

Ec_{50} cAMP assay: $5.61x10^{-9}$ M

Ec_{50} Calcium assay: $2.38x10^{-9}$ M

Z'_{cAMP}: 0.85+/- 0.01

$Z'_{Calcium}$: 0.81+/- 0.01

VAT No. ESB95481909

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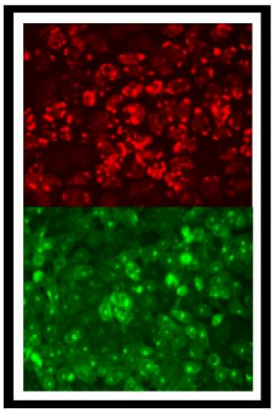

Product Name: ENDRB Multiplexed Nomad cell line
Reference: P70752
Recp. Official Full Name: Endothelin B receptor
DNA Accession Number: AY275463
Host Cell: U2OS
Resistance: G418 + Puromycin + Hygromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of Multiplexed Nomad ENDRB contains U2OS cells stably expressing cAMP Nomad-FP650 biosensor, Calcium Nomad-tGFP biosensor and Endothelin receptor B (with no tag).

Innoprot Multiplexed Nomad ENDRB cell line has been designed to assay compounds or analyze their capability to modulate Endothelin receptor B. When an agonist binds to ENDRB, a G protein is activated, which in turn triggers a cellular response mediated by cAMP and calcium.

This cell line has been validated measuring cAMP and calcium increase in the cytosol analyzing cAMP and calcium biosensors intensity and distribution within the cell.

This highly reproducible assay has been validated using Endothelin as agonist and measuring by both fluorescence intensity and image analysis.

About Nomad Biosensor Family

Nomad Biosensor family is based in a fluorescent polypeptide that in the presence or absence of cAMP or calcium changes its localization within the cell.

Before the stimulation mediated by the agonist of interest, 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 Nomad Biosensor that promotes its cellular relocation in the vesicular trafficking of the cells (cAMP) or an increase in the fluorescence (calcium).

In a cell line co-expressing Multiplexed Nomad Biosensor and a GPCR, the activity can be easily quantified on living cells by image analysis or fluorescence emission in a microplate reader.

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cAMP Measurement

Multiplexed Nomad U2OS cells, stably expressing label-free Endothelin receptor type B (ENDRB) and both Nomad biosensors red cAMP & Green Ca++, were stimulated with 8 log dilution series ranging from 0 to 1 µM of Endothelin during 24h (n=5). % Activity was calculated relative to positive (1µM).

Image analysis

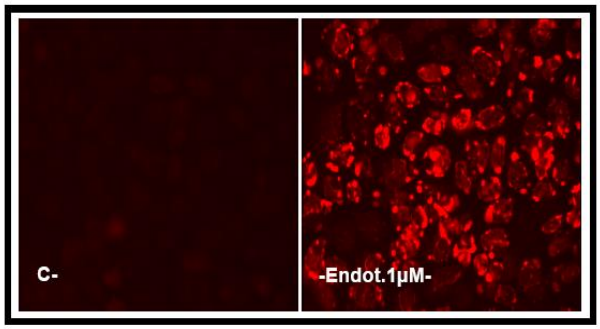

Fig1. Multiplex ENDRB Nomad cell line in basal conditions (left) and stimulated with endothelin (right)

Fluorescence intensity analysis

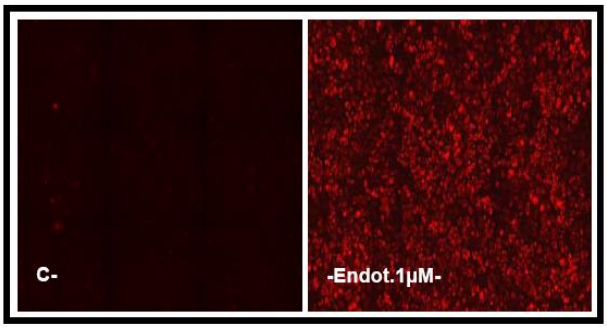

Fig2. Multiplex ENDRB Nomad cell line in basal conditions (left) and stimulated with endothelin (right)

Activation and biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The **EC50** for the Endothelin was ~ **5.61x10^{-9}M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.85**+/-0.02.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The **EC50** for the Endothelin was ~ **2.83x10^{-9}M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.78**+/-0.02.

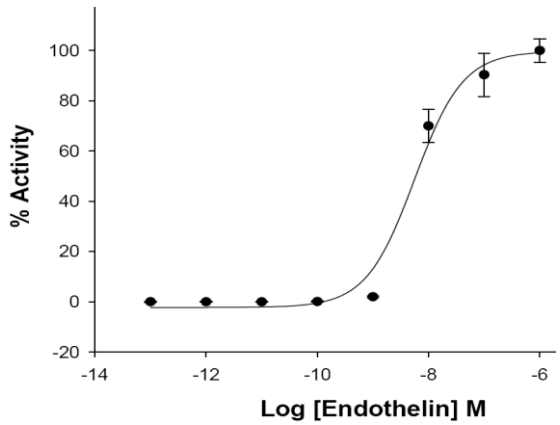

Fig3. Concentration response curve for Endothelin in Multiplexed Nomad ENDRB cell line analyzed using a high-content bioimager.

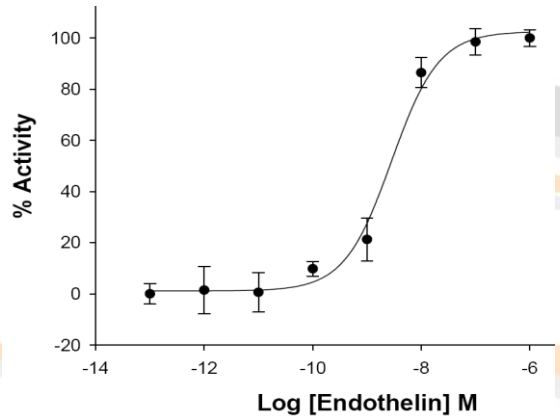

Fig4. Concentration response curve for Endothelin in Multiplexed Nomad ENDRB cell line analyzed using a fluorescence microplate reader.

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Ca^{++} Measurement

Multiplexed Nomad U2OS cells, stably expressing label-free Endothelin receptor type B (ENDRB) and both Nomad biosensors Red-cAMP & Green-Ca++, were stimulated with 8 log dilution series ranging from 0 to 1 µM of Endothelin during 24h (n=5). % Activity was calculated relative to positive (1µM).

Image analysis

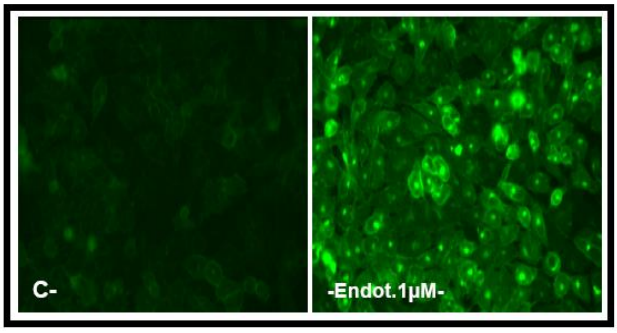

Fig1. Multiplex ENDRB Nomad cell line in basal conditions (left) and stimulated with endothelin (right).

Fluorescence intensity analysis

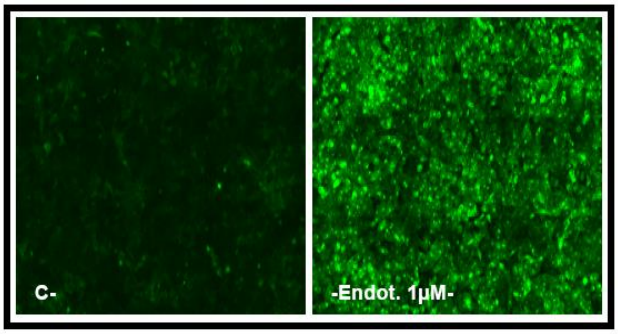

Fig2. Multiplex ENDRB Nomad cell line in basal conditions (left) and stimulated with endothelin (right).

Activation and biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The **EC50** for the Endothelin was ~ **$2.38x10^{-9}$M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.81**+/-0.02.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The **EC50** for the Endothelin was ~ **$2.46x10^{-9}$M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.91**+/-0.02.

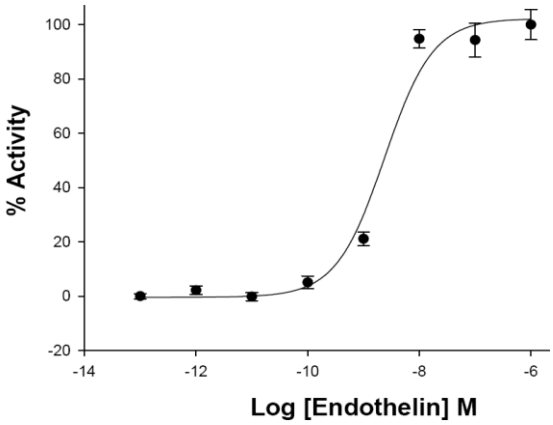

Fig2. Dose response curve for Endothelin in Multiplexed Nomad ENDRB cell line analyzed using a high-content bioimager.

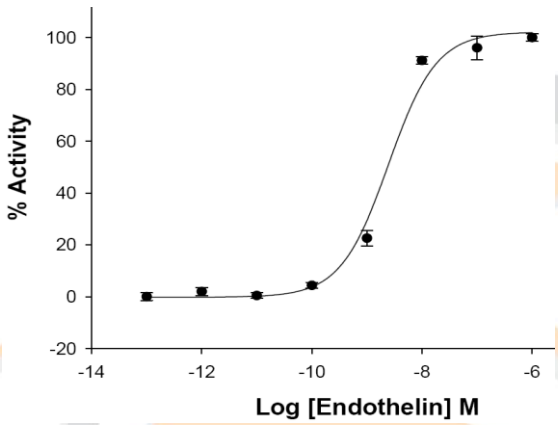

Fig2. Dose response curve for Endothelin in Multiplexed Nomad ENDRB cell line analyzed using a fluorescence microplate reader.

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