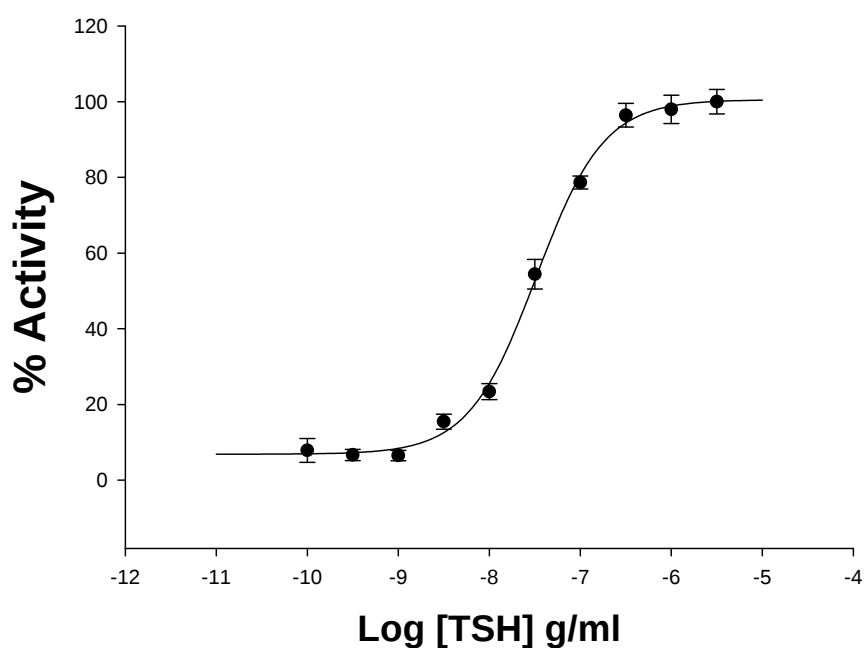
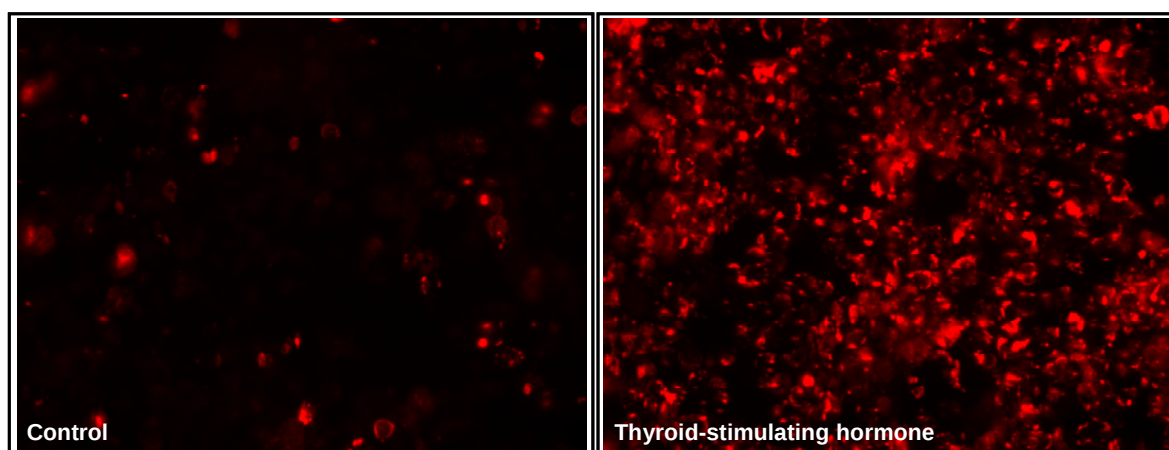


cAMP NOMAD-FP650 cell lines

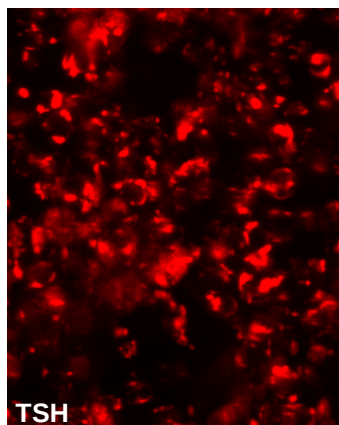
-THYROID-STIMULATING HORMONE RECEPTOR (TSHR)-



Red cAMPNomad-TSHR (HEK293 cell line)

EC₅₀ Thyroid-stimulating hormone (TSH): 3.27×10^{-8} g/ml

Z': 0.81 $\pm$ 0.01



Product Name: TSHR _{cAMP}Nomad cell line

Reference: P70599

Recp. Official Full Name: Thyroid-stimulating hormone receptor

DNA Accession Number: AY429111

Host Cell: HEK293

Resistance: G418 + Puromycin

Quantity: > 3 x 10⁶ cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of _{cAMP}Nomad TSHR contains HEK293 cells stably expressing _{cAMP}Nomad-FP650 biosensor and thyroid-stimulating hormone receptor (with no tag).

Innoprot _{cAMP}Nomad TSHR cell line has been designed to assay compounds or analyze their capability to modulate Thyroid-stimulating hormone receptor. When an agonist binds to TSHR 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 _{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 thyroid-stimulating hormone as agonist in a High Content Analysis (HCA) and a High Throughput Analysis (HTA).

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.



cAMP Assay

Red_{cAMP}Nomad HEK293 cells, stably expressing Thyroid-stimulating Hormone Receptor (TSHR), were stimulated with 9 log dilution series ranging from 0 to 3 µg/mL of thyroid stimulating hormone during 24h (n=8). % Activity was calculated relative to positive (3 µg/mL).

Fluorescence intensity analysis

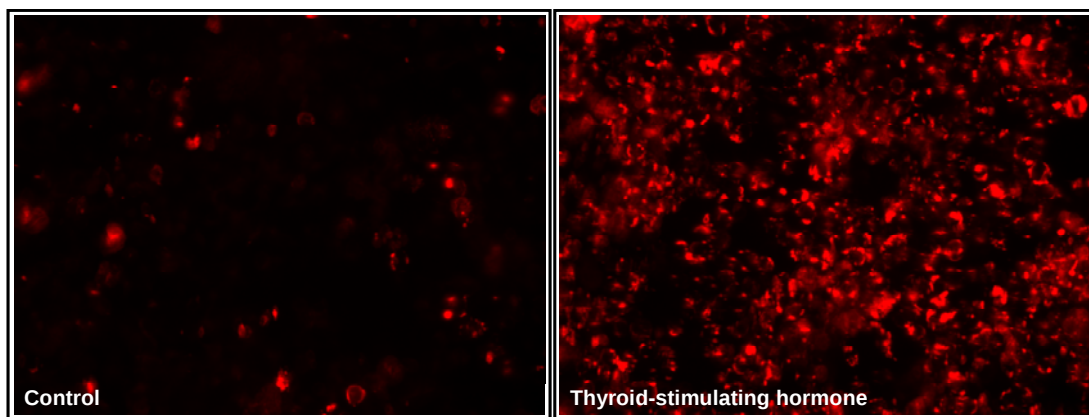


Fig1. Red_{cAMP}Nomad biosensor negative control and TSH stimulation.

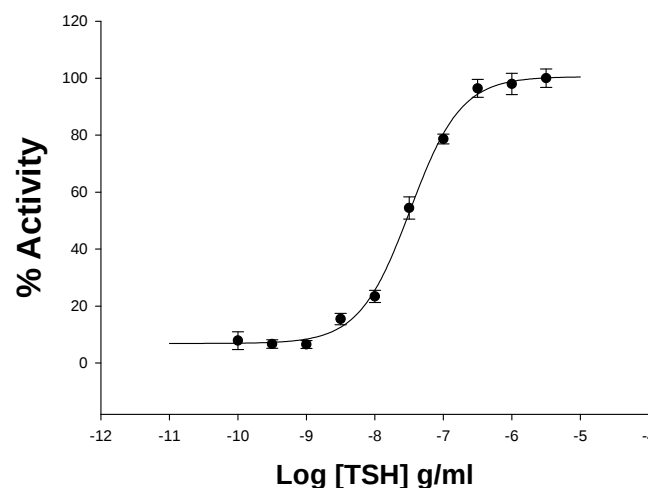


Fig 2. Concentration-response curve for thyroid stimulating hormone in Red_{cAMP}Nomad-TSHR cell line analyzed using “Synergy 2” microplate reader from Biotek. The EC₅₀ for TSH was 3.27×10^{-8} g/ml after a treatment of 24 h with the agonist. The assay was validated with an average of $Z' = 0.81 \pm 0.01$.