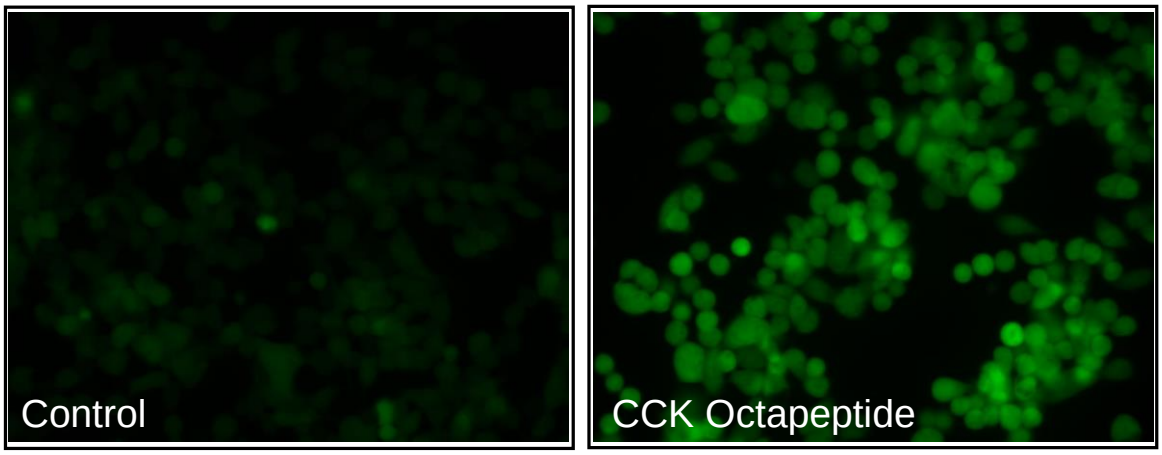
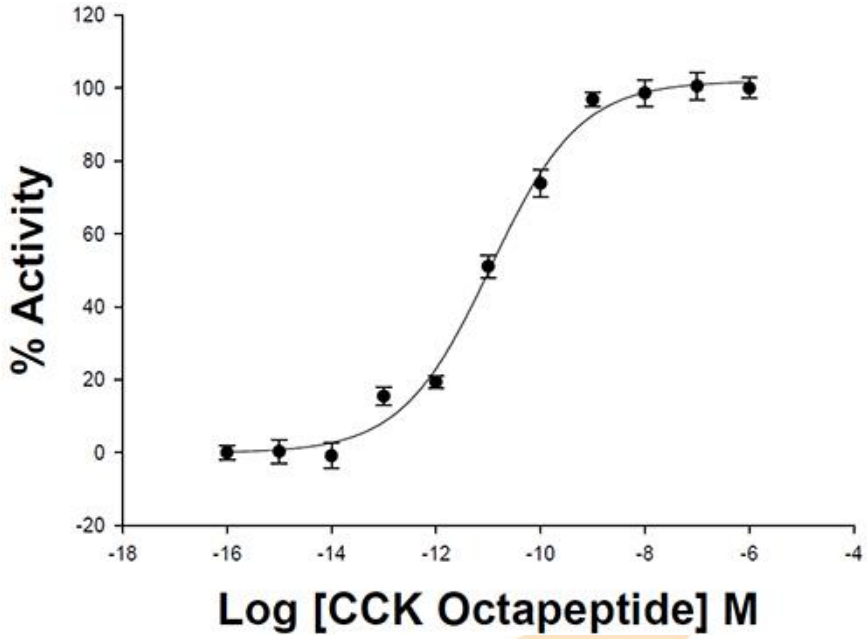
REF: P70808

Arrestin-NOMAD Cell Lines

-CHOLECYSTOKININ B RECEPTOR (CCKBR)-





Green $_{Arrest}$Nomad-CCKBR (HEK293 cell line)

Ec_{50} CCK Octapeptide: $1.12x10^{-11}$ M

Z´: 0.86+/- 0.01

VAT No. ESB95481909

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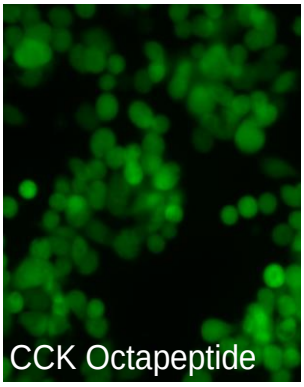

Product Name: CCKBR ArresNomad cell line

Reference: P70808

Recp. Official Full Name: Cholecystokinin B receptor

DNA Accession Number: AY322551

Host Cell: HEK293

Resistance: Hygromycin + Puromycin

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of green ArresNomad-CCKBR contains U2OS cells stably expressing green ArresNomad biosensor and Cholecystokinin B receptor (with no tag).

Innoprot´s green ArresNomad-CCKBR cell line has been designed to assay compounds or analyze their capability to modulate Cholecystokinin B receptor. When an agonist binds to CCKBR a G protein is activated which, in turn, triggers a cellular response mediated by calcium and a subsequent internalization mediated by ß-Arrestin.

This cell line has been validated measuring Arrestin-mediated internalization analyzing green ArresNomad 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 CCK Octapeptide as agonist in a Fluorescence Microplate Reader.

About Green ArresNomad Biosensor

Green ArresNomad Biosensor is a fluorescent polypeptide that measures fluctuations in Arrestin signalling pathway changing its localization and fluorescent intensity emission within the cell.

An increase in the second messenger concentration leads to a change in the structural folding of Nomad Biosensor that promotes a conformational change and an increase in the fluorescence.

In a cell line co-expressing Green ArresNomad 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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ß-Arrestin Assay

Green $_{Arres}$Nomad HEK293 cells, stably expressing Cholecystokinin B Receptor (CCKBR), were stimulated with 9 log dilution series ranging from 0 to 1 µM of CCK Octapeptide during 24h (n=8).
% Activity was calculated relative to positive (1 µM).

Fluorescence intensity analysis

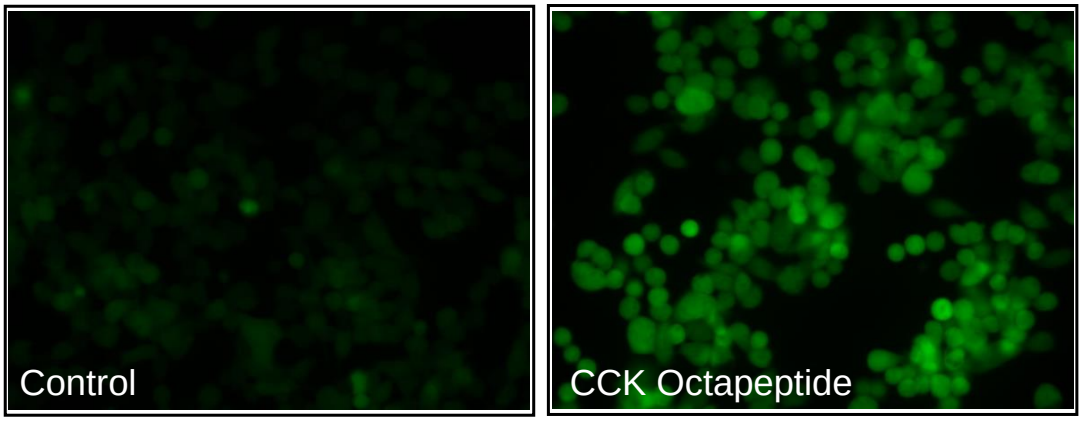

Fig 1. Green $_{Ar}$Nomad biosensor negative control and CCK Octapeptide stimulation.

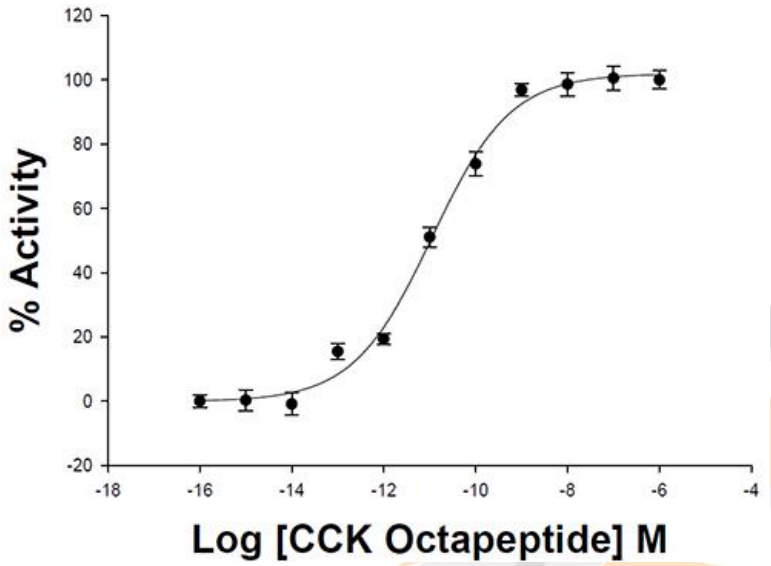

Fig 2. Concentration-response curve for CCK Octapeptide in Green $_{Arres}$Nomad-CCKBR cell line analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for CCK Octapeptide was $1.12x10^{-11}$ M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.86+/-0.01.

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