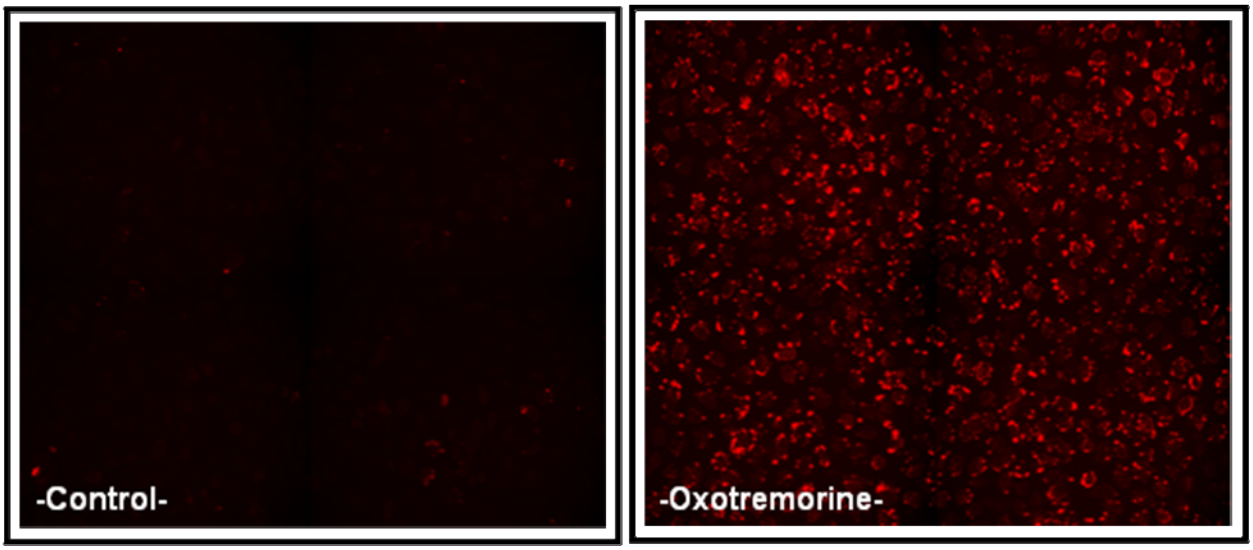
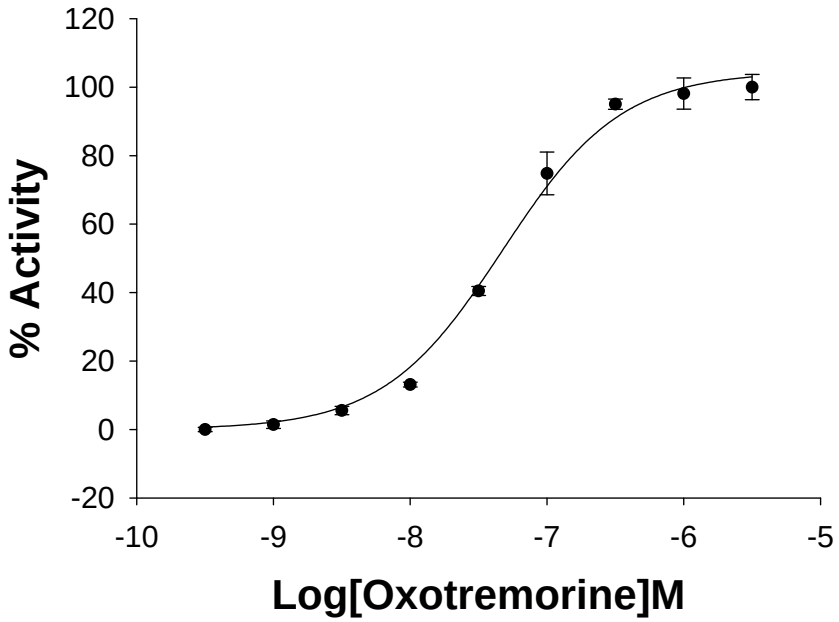
REF: P70545

PKANOMAD-FP650 CELL LINES

-MUSCARINIC ACETYLCHOLINE RECEPTOR M4 (CHRM4)-





Red PKANomad-CHRM4 (U2OS cell line)

Ec50 Oxotremorine: 4.72 x 10^{-8} M

Z´: 0.87+/- 0.01

VAT No. ESB95481909

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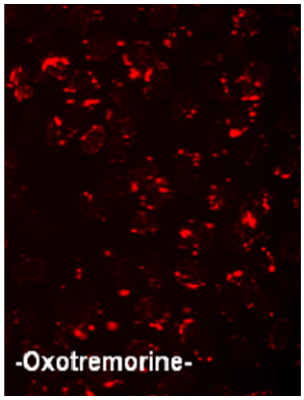


Product Name: CHRM4 $_{PKA}$Nomad cell line
Reference: P70545
Recp. Official Name: Muscarinic Acetylcholine Receptor M4
DNA Accession Number: X15265
Host Cell: U2OS
Resistance: G418 + Puromycin
Quantity: > 3 x 10^6 cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of red $_{PKA}$Nomad-CHRM4 contains U2OS cells stably expressing red $_{PKA}$Nomad biosensor and Muscarinic Acetylcholine Receptor M4 (with no tag).

Innoprot´s red $_{PKA}$Nomad-CHRM4 cell line has been designed to assay compounds or analyze their capability to modulate Muscarinic Acetylcholine Receptor M4. When an agonist binds to CHRM4, Gi/o is activated, which in turn, triggers a cellular response involving PKA activation. Activated PKA interacts with $_{PKA}$Nomad Biosensor, increasing its fluorescence intensity.

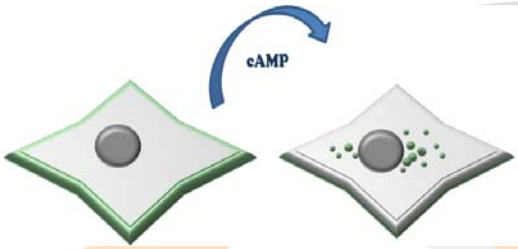
This cell line has been validated measuring PKA activation in the cytosol analyzing both red $_{PKA}$Nomad biosensor intensity and distribution within the cell.

This highly reproducible assay has been validated using Oxotremorine as agonist analyzing both fluorescence intensity changes and biosensor redistribution.

About Red $_{PKA}$Nomad Biosensor

Red $_{PKA}$Nomad Biosensor is a fluorescent polypeptide that changes its localization within the cell when PKA is activated, increasing also its own fluorescence intensity in a dose-response manner.

Before PKA activation, the fluorescent biosensor is localized in the cellular membrane. Activated PKA interacts with $_{PKA}$Nomad Biosensor leading to a change in its the structural folding promoting its cellular relocation in the vesicular trafficking of the cells.



In a cell line co-expressing red $_{PKA}$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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PKA activation Assay

PKANomad U2OS cells, stably expressing Muscarinic Acetylcholine Receptor M4 (CHRM4), were stimulated with 9 half log dilution series ranging from 0 to 3 µM of Oxotremorine during 24h (n=4). %Activity was calculated relative to positive (3 µM).

Image analysis

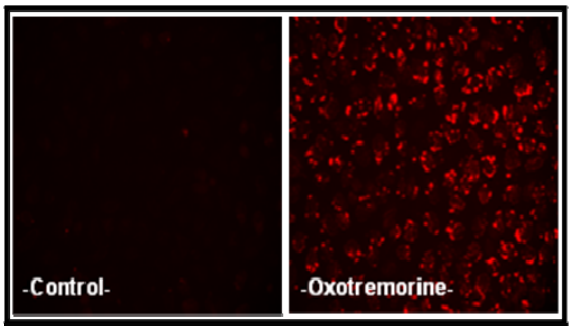

Fig1. Red PKANomad biosensor negative control and Oxotremorine stimulation.

Activation and biosensor change of localization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences. The EC50 for Oxotremorine was ~ $4.72x10^{-8}$ M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.87+/-0.01.

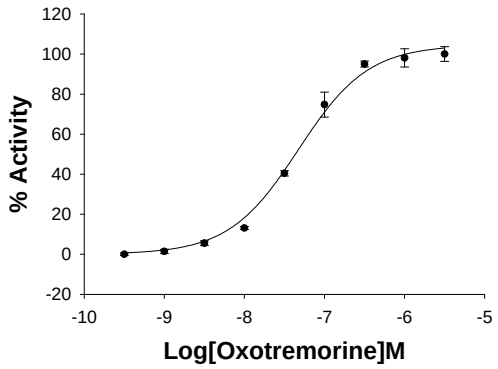

Fig2. Concentration response curve for Oxotremorine in Red PKANomad-CHRM4 cell line analyzed using a high-content bioimager.

Fluorescence intensity analysis

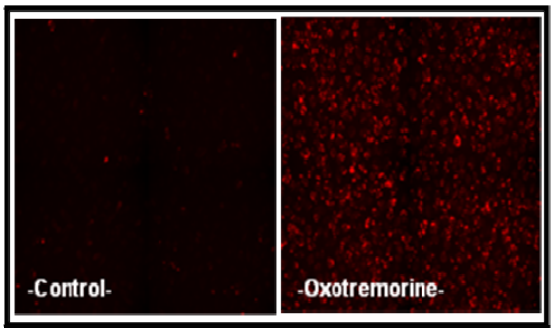

Fig3. Red PKANomad biosensor negative control and Oxotremorine stimulation.

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The EC50 for Oxotremorine was ~ $5.89x10^{-8}$ M after a treatment of 24 h with the agonist. The assay was validated with an average of Z´= 0.81+/-0.01.

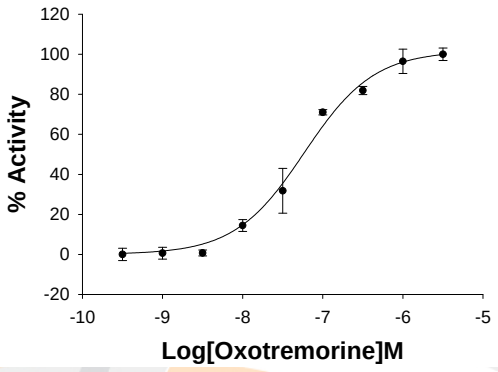

Fig4. Concentration response curve for Oxotremorine in Red PKANomad-CHRM4 cell line analyzed using a microplate reader.

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