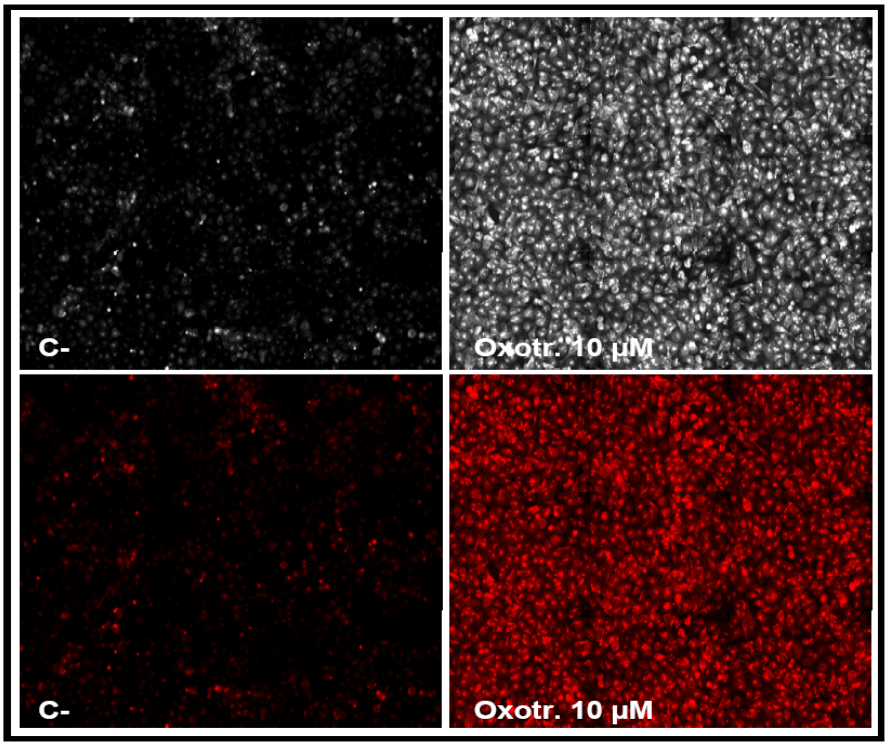
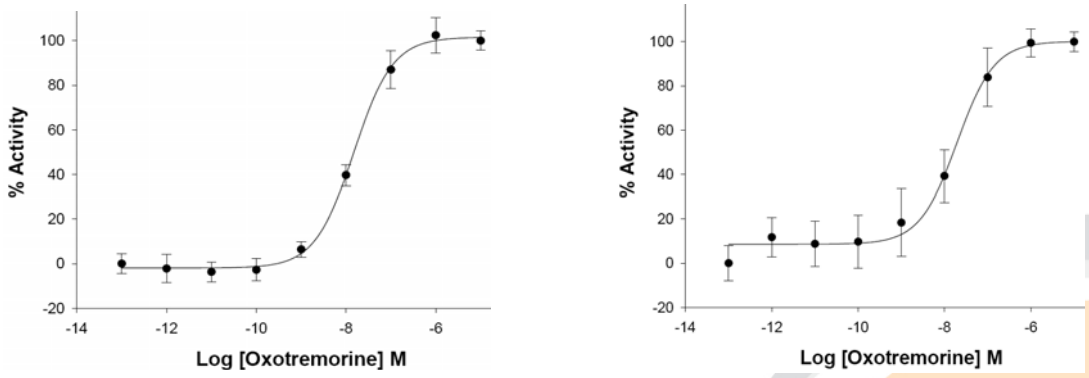
REF: P70603

# DAGNOMAD CELL LINES

## - MUSCARINIC ACETYLCHOLINE RECEPTOR M5-




### DAGNomad M5 Cell Line

**$Ec_{50}$ Image assay:** 1.48 x $10^{-8}$ M **$Ec_{50}$ Fluor. Intens. assay:** 1.95x$10^{-7}$ M

**$Z'_{Image}$:** 0.74+/- 0.01 **$Z'_{FI}$:** 0.63+/- 0.01

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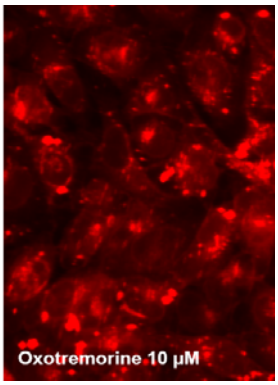


**Product Name:** $_{DAG}$NOMAD M5 Cell Line
**Reference:** P70603
**DNA Accession Number:** Gene Bank M80333
**Recp. Official Full Name:** Muscarinic acetylcholine receptor M5
**Host Cell:** $_{DAG}$Nomad U2OS Cell Line
**Fluorescence:** excitation/emission maxima at 574/602 nm
**Resistances:** Puromycin & G418
**Quantity:** > 3 x $10^6$ cells / vial
**Storage:** Liquid Nitrogen

## *Assay Briefly description*

Each vial of $_{DAG}$NOMAD M5 Cell Line contains 3 million U2OS cells stably expressing $_{DAG}$Nomad biosensor and human M5 receptor.

Innoprot $_{DAG}$NOMAD M5 Cell Line has been designed to assay compounds or analyze their capability to modulate M5 receptor. When the agonist binds toM5 a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (DAG).

This cell line has been validated measuring DAG increase in the cytosol analyzing $_{DAG}$NOMAD biosensor fluorescence increase 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 Oxotremorine as a M5 agonist in a High Content Analysis (HCA) and in a fluorescence plate reader.

## *About Nomad $_{DAG}$NOMAD Biosensor*

$_{DAG}$NOMAD Biosensor is a red fluorescent polypeptide that in the presence or absence of DAG increases the fluorescence intensity in the cell. An increase in this second messenger concentration leads to a change in the structural folding of Nomad Biosensor that promotes its cellular fluorescence intensity. In a cell line co-expressing the $_{DAG}$NOMAD Biosensor and a GPCR, after the receptor activation using its agonist, the activity can be easily quantified on living cells by fluorescence increase signal.

$_{DAG}$NOMAD Biosensor possesses true-red fluorescence (with excitation/emission maxima at 574/602 nm, respectively), optimal for detection via most popular filter sets, and is easily distinguished from background signals

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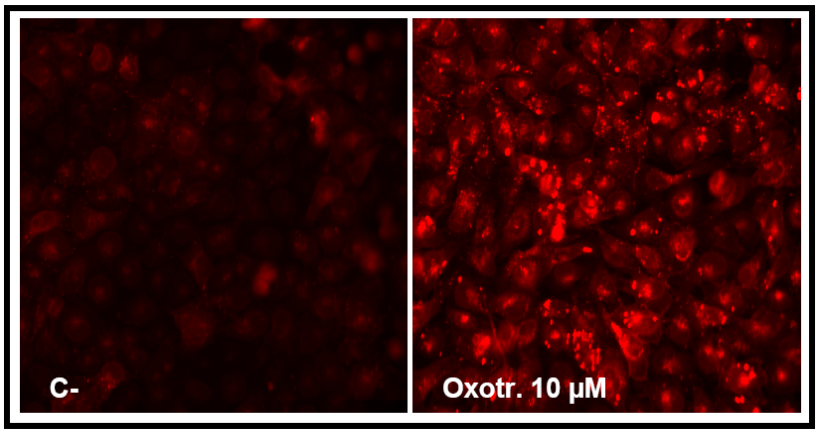
# FUNCTIONAL CHARACTERIZATION

$_{DAG}$Nomad M5 cells were stimulated with 9 log dilution series ranging from 0 to 10 µM of Oxotremorine during 24h (n=5). % Activity was then calculated relative to positive (10µM).




### *Image analysis*

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 Oxotremorine was ~ **1.48x10$^{-8}$M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.74**+/-0.02.

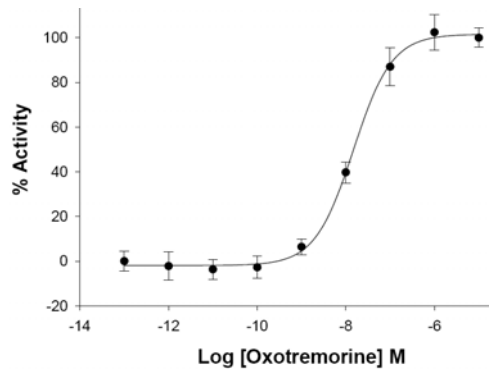



Fig1. Concentration response curve for Oxotremorine in Nomad-BioDAG-FP602 M5 cell line analyzed using a high-content bioimager.

### *Fluorescence intensity analysis*

The increase in the fluorescence was detected and analyzed using "Synergy 2" microplate reader from Biotek. The **EC50** for the Oxotremorine was ~ **1.95x10$^{-7}$M** after a treatment of 24 h with the agonist.The assay was validated with an average of **Z´= 0.63**+/-0.02.

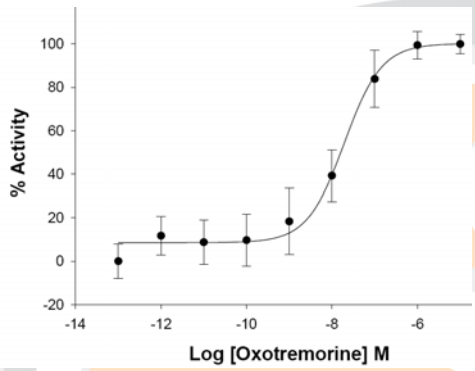



Fig2. Concentration response curve for Oxotremorine in Nomad-BioDAG-FP602 M5 cell line analyzed using a fluorescence microplate reader.

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