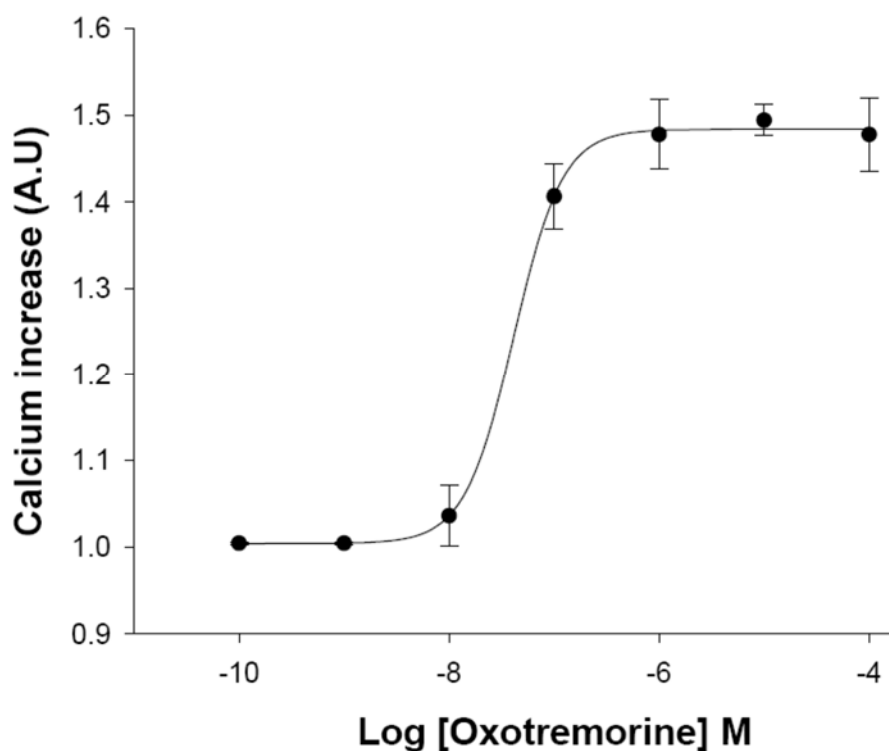


## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

### MUSCARINIC M<sub>3</sub> RECEPTOR CELL LINE



**Product name:** Muscarinic acetylcholine receptor M3 / U2OS cell line

**EC<sub>50</sub> Oxotremorine:**  $4.11 \times 10^{-8}$  M

**Z':** 0.72 $\pm$  0.02

## MUSCARINIC M<sub>3</sub> RECEPTOR CELL LINE

<b>Product Name:</b>	CHRM3 / U2OS
<b>Official Full Name:</b>	Muscarinic acetylcholine receptor M3
<b>DNA Accesion Number:</b>	GenBank: X15266
<b>Host Cell:</b>	U2OS
<b>Format:</b>	cryopreserved vials
<b>Resistance:</b>	G418
<b>Size:</b>	<i>P30144</i> : 2 vials of 3 x 10 <sup>6</sup> proliferative cells <i>P30144-DA</i> : 1 vial of 2.5x10 <sup>6</sup> division-arrested cells
<b>Storage:</b>	Liquid Nitrogen

### Assay Briefly description

Each vial of HiTSeeker M<sub>3</sub> contains U2OS cells stably expressing human Muscarinic acetylcholine receptor M<sub>3</sub> with no tag.

Innopro M<sub>3</sub> cell line has been designed to assay compounds or analyze their capability to modulate Muscarinic acetylcholine receptor M<sub>3</sub>. When the agonist binds to M<sub>3</sub> a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium).

This cell line has been validated measuring calcium increase in the cytosol. The high reproducibility of this assay allows monitoring M<sub>3</sub> activation process in High Throughput Screening.

### About M<sub>3</sub>

Muscarinic acetylcholine receptors are G protein-coupled receptors. M<sub>1</sub>, M<sub>3</sub>, M<sub>5</sub> receptors couple to G proteins of the G<sub>q/11</sub> family, which activate phospholipase C.

M<sub>2</sub> and M<sub>4</sub> receptors couple to G<sub>i/o</sub>-type G proteins that inhibit adenylyl cyclase activity. Muscarinic receptors control many effects of acetylcholine in the central and peripheral nervous system.

M<sub>3</sub> receptor is found in smooth muscles, the endocrine glands, the exocrine glands, as well as the lungs. It is also found in the CNS, where it induces emesis. In general, it causes smooth muscle contraction and increased glandular secretions.

M<sub>3</sub> receptor is thought to be implicated in Alzheimer's disease.

## Assay Characterization

Our expression plasmid contains the coding sequence of human M3 protein. Our plasmid was transfected in U2OS cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

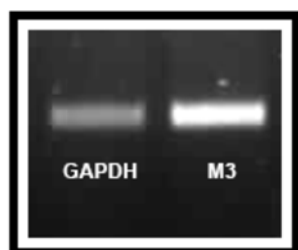


Fig.1. GAPDH housekeeping gene and M3 RT-PCR.

## Validation of M3 cell line

### Calcium assay ( $EC_{50} = 4.11 \times 10^{-8} M$ )

A typical fluorescent calcium assay was performed using Fura-2/AM ratiometric. Calcium increase inside the cell was measured using the ratio of the fluorescence from Fura2 bound and not bound to the ion. Image acquisition was performed using a “BD Pathway 855” High-Content Bioimager from BD Biosciences.

Cells were incubated with Fura2-AM and treated with increasing Oxotremorine concentrations.

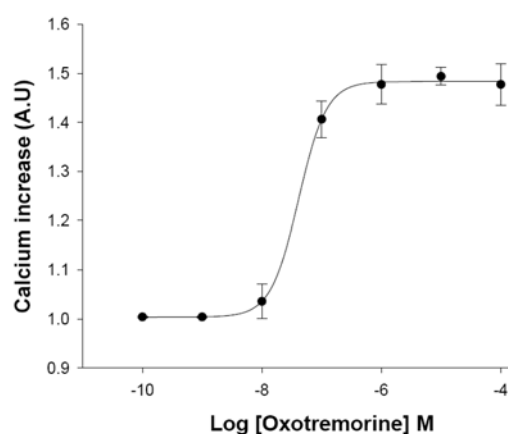


Fig.2. M3 dose response in calcium assay. Cells were treated with Oxotremorine concentrations ranging from 0 to 1 mM, n=5. The  $EC_{50}$  for Oxotremorine was  $\sim 4.11 \times 10^{-8} M$ . The calcium assay was validated with a  $Z' = 0.72 \pm 0.02$  for High Content Screening.