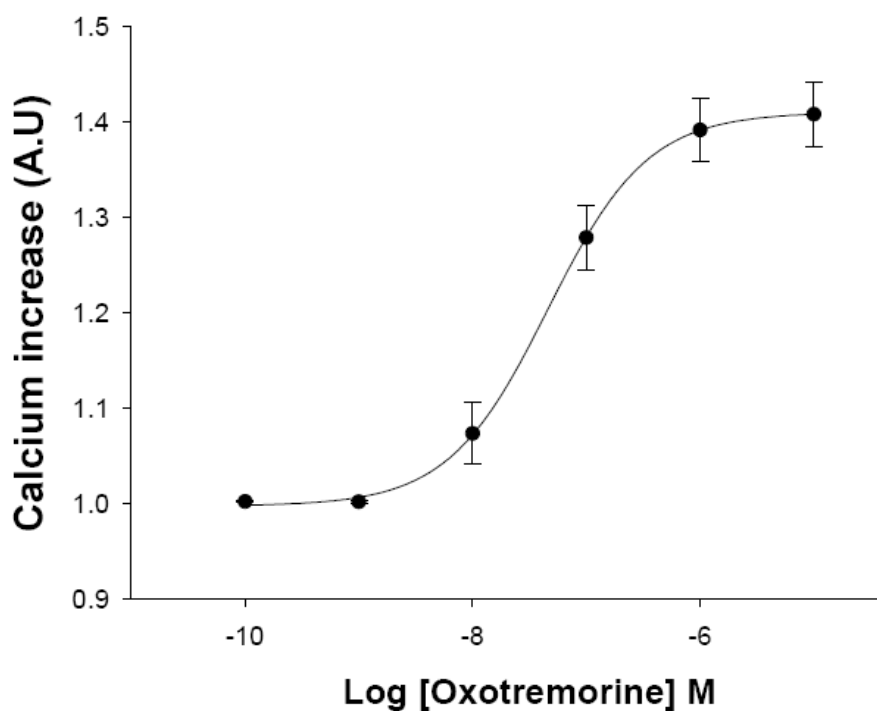


HiTSeeker CELL LINES (LABEL-FREE GPCRS)
- MUSCARINIC ACETYLCHOLINE RECEPTOR M5 CELL LINE -



Product name: Muscarinic acetylcholine receptor M5 (M5) /U2OS cell line

Ec₅₀ Oxotremorine: 4.5×10^{-8} M

Z': 0.74 \pm 0.02

- MUSCARINIC ACETYLCHOLINE RECEPTOR M5 U2OS CELL LINE -

Product Name:	M5 (CHRM5)/U2OS
Official Full Name:	Muscarinic acetylcholine receptor M5
DNA Accesion Number:	GenBank: M80333
Host Cell:	U2OS
Format:	2 cryopreserved vials
Resistance:	G418
Size:	<i>P30146</i> : 2 vials of 3×10^6 proliferative cells <i>P30146-DA</i> : 1 vial of 2.5×10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker-CHRM5 contains U2OS cells stably expressing human Muscarinic acetylcholine receptor M5 with no tag.

Innoprot M5 cell line has been designed to assay compounds or analyze their capability to modulate Muscarinic acetylcholine receptor M5. When the agonist binds to M5 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 M5 activation process in High Throughput Screening.

About M5

Muscarinic acetylcholine receptors are G protein-coupled receptors. M1, M3, M5 receptors couple to G proteins of the $G_q/11$ family, which activate phospholipase C.

M2 and M4 receptors couple to $G_{i/o}$ -type G proteins that inhibit adenylyl cyclase activity. Muscarinic receptors control many effects of acetylcholine in the central and peripheral nervous system.

The clinical implications of this receptor have not been fully explored; however, stimulation of this receptor is known to effectively decrease cAMP levels and downregulate the activity of protein kinase A (PKA).

Assay Characterization

Our expression plasmid contains the coding sequence of human M5 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).

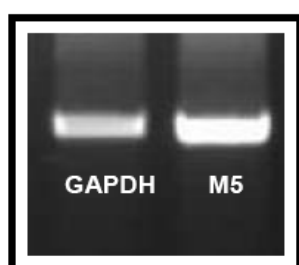


Fig.1. GAPDH housekeeping gene and M5 RT-PCR.

Validation of M5 cell line

Calcium assay ($EC_{50} = 4.5 \times 10^{-8} \text{ M}$)

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.

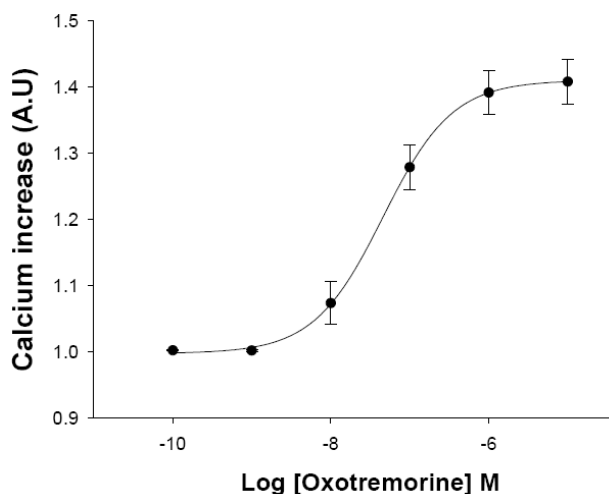


Fig.2. M5 dose response in calcium assay. Cells were treated with **Oxotremorine** concentrations ranging from 0 to 10 μM , $n=5$. The EC_{50} for **Oxotremorine** was $\sim 4.5 \times 10^{-8} \text{ M}$. The calcium assay was validated with a $Z' = 0.74 \pm 0.02$ for High Content Screening.