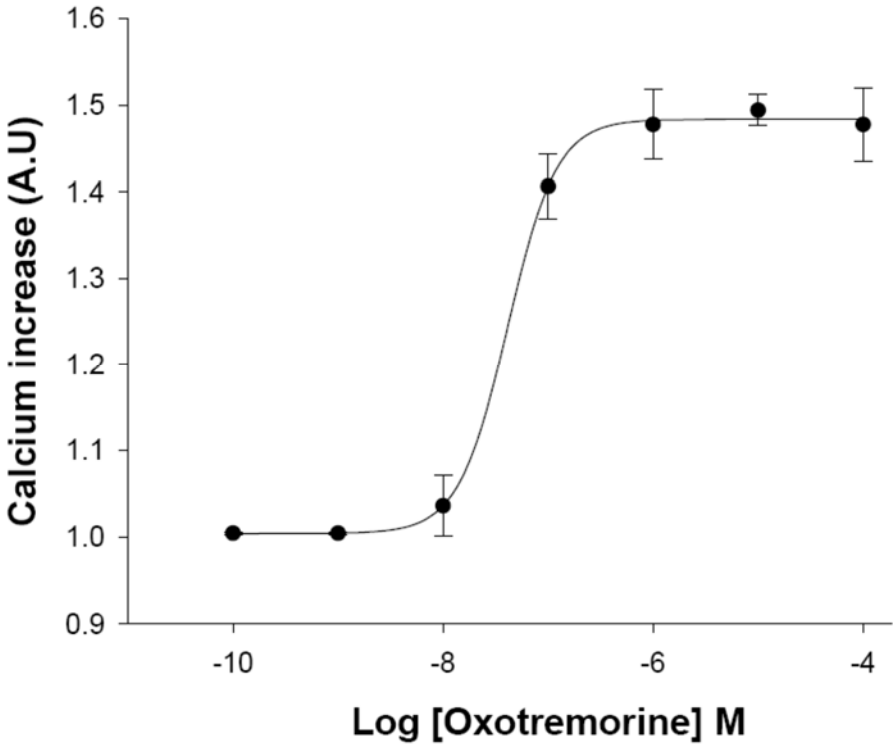
REF: P30144

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

MUSCARINIC M_3 RECEPTOR CELL LINE



Product name: Muscarinic acetylcholine receptor M3 / U2OS cell line

Ec_{50} Oxotremorine: 4.11 x 10^{-8} M

Z´: 0.72+/- 0.02

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MUSCARINIC M_3 RECEPTOR CELL LINE

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

Assay Briefly description

Each vial of HiTSeeker M3 contains U2OS cells stably expressing human Muscarinic acetylcholine receptor M3 with no tag.

Innoprot M3 cell line has been designed to assay compounds or analyze their capability to modulate Muscarinic acetylcholine receptor M3. When the agonist binds to M3 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 M3 activation process in High Throughput Screening.

About M3

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.

M_3 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.

M3 receptor is thought to be implicated in Alzheimer´s disease.

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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).

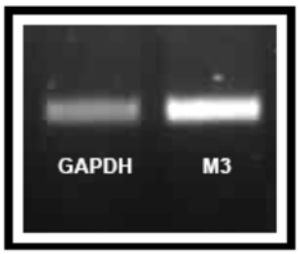

Fig.1. GAPDH housekeeping gene and M3 RT-PCR.

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

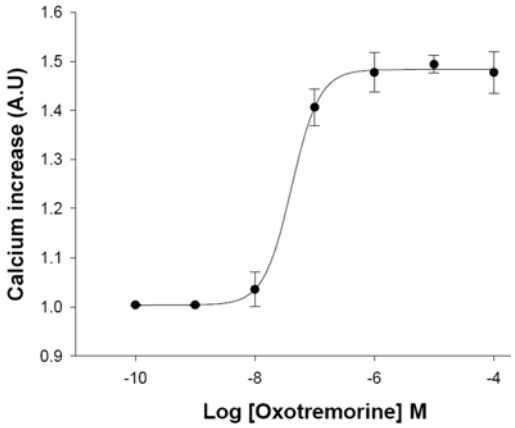

Fig.2. M3 dose response in calcium assay. Cells were treated with **Oxotremorine** concentrations ranging from 0 to 1 mM, n=5. The EC50 for **Oxotremorine** was ~**$4.11x10^{-8}$ M.** The calcium assay was validated with a Z´= 0.72+/- 0.02 for High Content Screening.

Validation of M3 cell line

Calcium assay (Ec50 = 4.11×10^{-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.

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