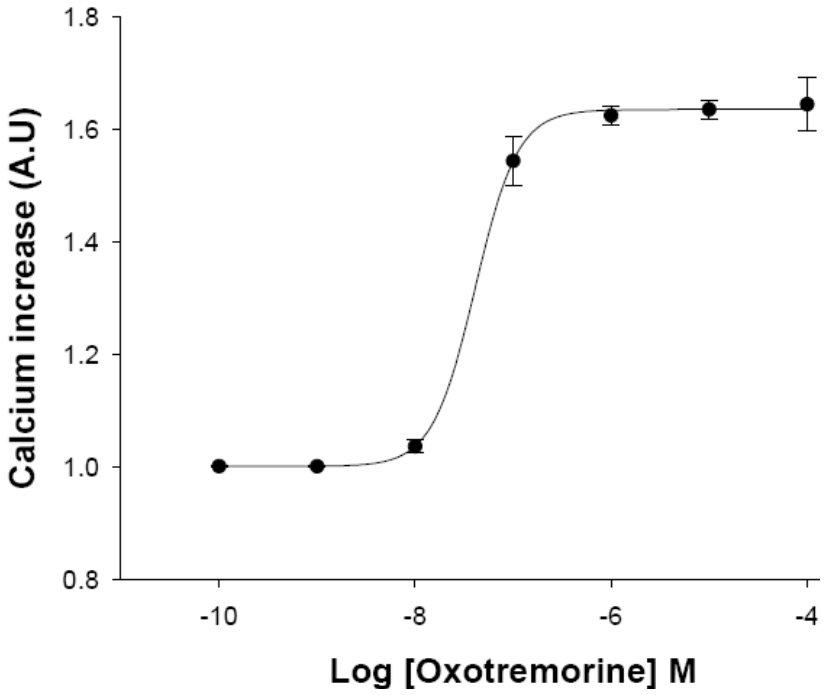
REF: P30142

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- MUSCARINIC ACETYLCHOLINE RECEPTOR M1 CELL LINE -



Product name: Muscarinic acetylcholine receptor M1 (M1) /U2OS cell line

Ec_{50} Oxotremorine: 4.08x 10^{-8} M

Z´: 0.78+/- 0.02

日本総代理店
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- MUSCARINIC ACETYLCHOLINE RECEPTOR M1 U2OS CELL LINE -

Product Name:	M1 (CHRM1)/U2OS
Official Full Name:	Muscarinic acetylcholine receptor M1
DNA Accession Number:	GenBank: BC007740
Host Cell:	U2OS
Format:	cryopreserved vials
Resistance:	G418
Size:	*P30142*: 2 vials of 3 x 10^6 proliferative cells *P30142-DA*: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial or HiTSeeker M1 contains U2OS cells stably expressing human Muscarinic acetylcholine receptor M1 with no tag.

Innoprot M1 cell line has been designed to assay compounds or analyze their capability to modulate Muscarinic acetylcholine receptor M1. When the agonist binds to M1 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 M1 activation process in High Throughput Screening.

About M1

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.

M1 receptor is known to mediate slow EPSP at the ganglion in the postganglionic nerve, and it is also found in exocrine glands and in the CNS. M1 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 M1 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).

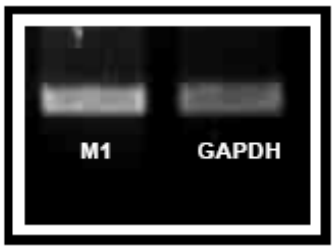

Fig.1. M1 and **GAPDH housekeeping gene RT-PCR.**

Validation of M1 cell line

Calcium assay (Ec50 = 4.08 x 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.

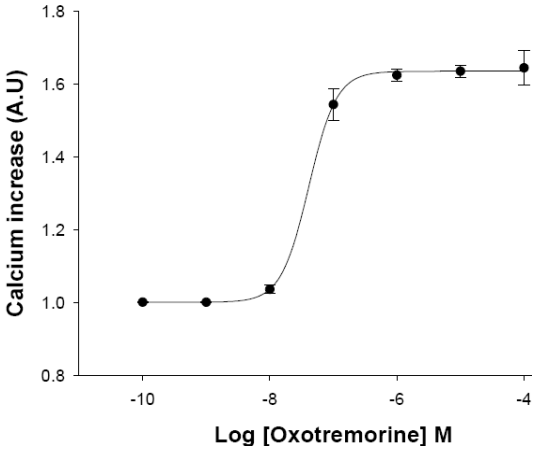

Fig.2. M1 dose response in calcium assay. Cells were treated with **Oxotremorine** concentrations ranging from 0 to 100 µM, n=5. The EC50 for **Oxotremorine** was ~**4.08x10^{-8}M**. The calcium assay was validated with a Z´= 0.78+/- 0.02 for High Content Screening.

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