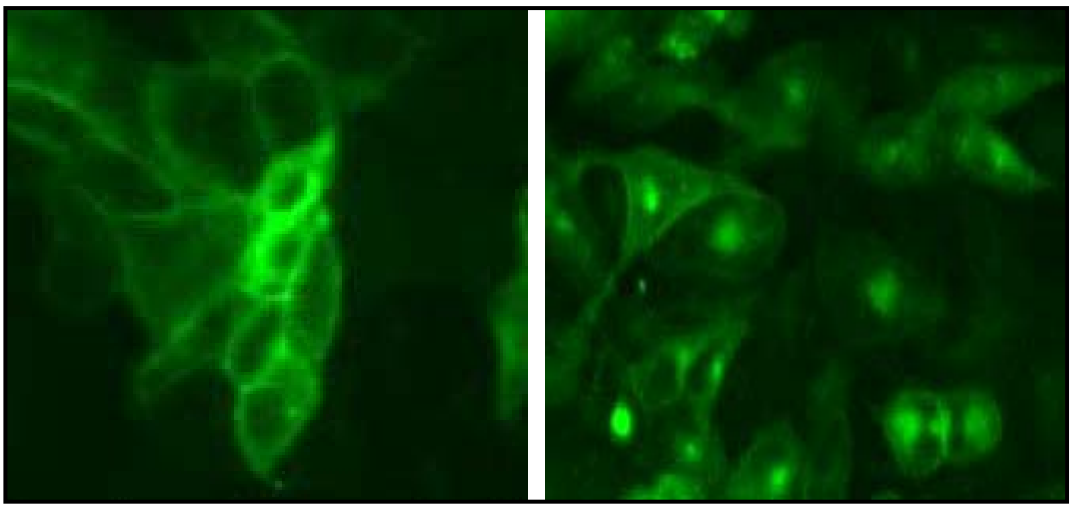
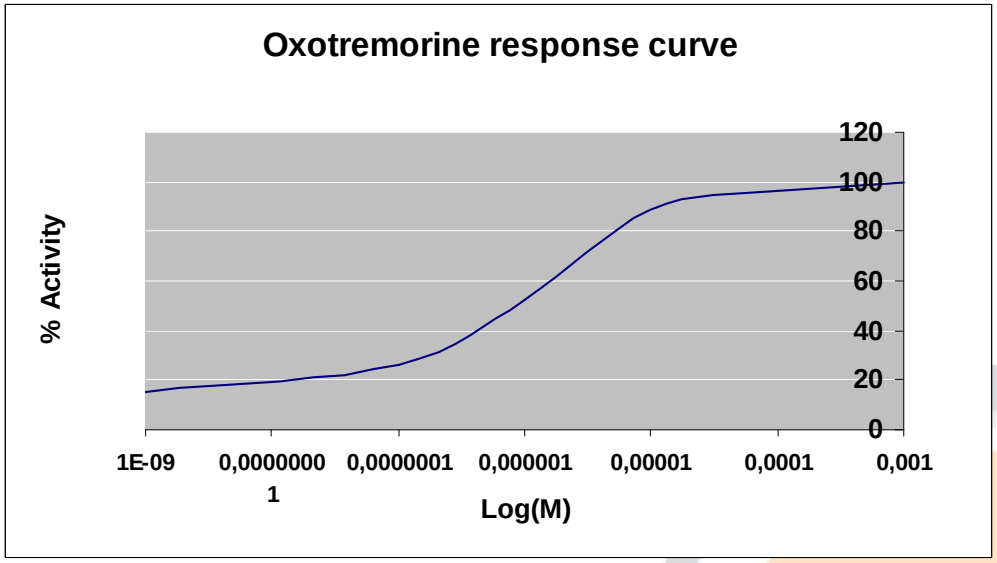
REF: P30204

RECEPTOR INTERNALIZATION ASSAYS

- HUMAN CHOLINERGIC RECEPTOR, MUSCARINIC 1 CELL LINE -





Product name: M1-tGFP / CHOK1 cell line

Ec_{50} oxotremorine: 1 x 10^{-6} M

Z´: 0.60 + /- 0.01

日本総代理店
オーラス・バイオサイエンス株式会社
〒271-0063 千葉県松戸市北松戸2-21-18
Tel.: 047-308-2204 | Fax: 047-308-2104
www.eolas-bio.co.jp

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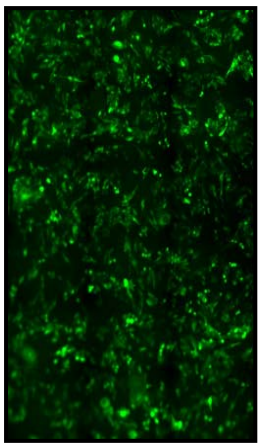
HUMAN CHOLINERGIC RECEPTOR, MUSCARINIC 1 CELL LINE



Product Name: CHRM1-tGFP/CHO-K1

Receptor Official Full Name: Cholinergic receptor, muscarinic 1

DNA Accesion Number: GenBank BC007740

Host Cell: CHO-K1

Format: 1 cryopreserved vial

Quantity: > 3 x 10^6 cells / vial

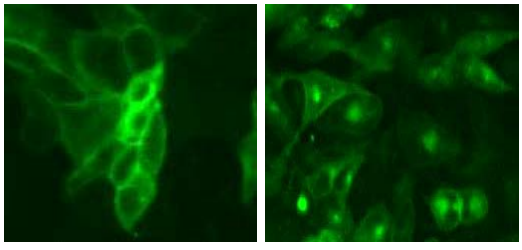
References:

- ***P30204:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30204-DA***: 1 vial of 2 x 10^6 division-arrested cells

Assay Briefly description

Each vial of CHRM1-tGFP/CHO-K1 contains CHO-K1 cells stably expressing human Acetylcholine receptor 1 (M1) tagged in the N-terminus with tGFP.

Innoprot M1 Internalizacion Assay has been designed to assay compounds or analyze stimuli for their ability to modulate cholinergic receptor, muscarinic 1 internalization process following and quantifying the fluorescence distribution inside the cells.



This highly reproducible assay allows monitoring CHRM1 receptor internalization process in High Content Analysis and fluorescence microscope applications.

About M1 Receptor

Cholinergic receptor, muscarinic 1. The muscarinic cholinergic receptors belong to a larger family of G protein-coupled receptors. The functional diversity of these receptors is defined by the binding of acetylcholine and includes cellular responses such as adenylate cyclase inhibition, phosphoinositide degeneration, and potassium channel mediation. Muscarinic receptors influence many effects of acetylcholine in the central and peripheral nervous system. The muscarinic cholinergic receptor 1 is involved in mediation of vagally-induced bronchoconstriction and in the acid secretion of the gastrointestinal tract. The gene encoding this receptor is localized to 11q13. The muscarinic acetylcholine receptor mediates various cellular responses, including inhibition of adenylate cyclase, breakdown of phosphoinositides and modulation of potassium channels through the action of G proteins.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Cholinergic muscarinic 1 receptor tagged in the N-terminal with tGFP protein. Our plasmid was transfected in CHO-K1 cells, using calcium phosphate method. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

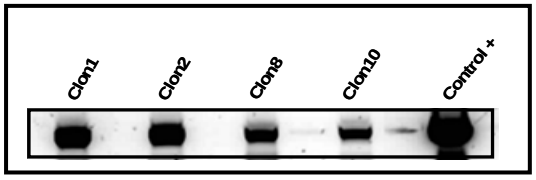

Fig.1. Clones CHMR1 mRNA expression.

Internalization assay for M1-tGFP

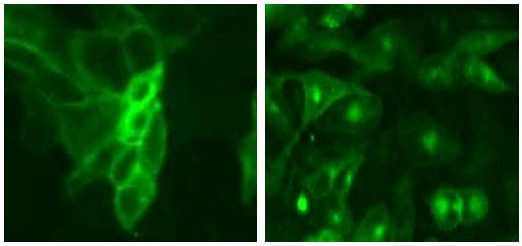

Fig.2. Internalization of M1-tGFP clone2 stimulated with Oxotremorine. Cells were treated with 1mM Oxotremorine for 24h. Internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

CHO-K1 stably expressing human Acetylcholine receptor 1 (M1) tagged in the N-terminus with tGFP were stimulated with different concentrations of Oxotremorine agonist during 24 hours. After that, the nucleus was stained with DAPI and M1 internalizated spots were detected by fluorescence using image analysis algorithms.

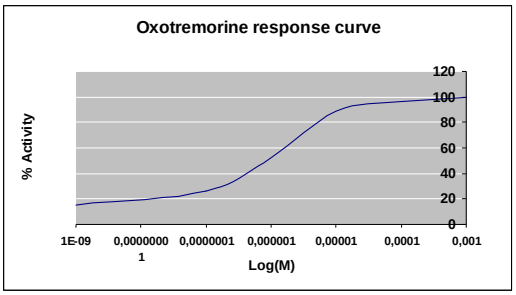

Fig.3. Oxotremorine concentrations response in the M1-tGFP internalization assay. Cells were treated with 8 log dilution series (n=4). The Ec50 for the oxotremorine was ~ 1uM after a treatment of 16h with agonist. Cells were fixed and the nucleus were stained with DAPI. % Activity was calculated relative to positive (1mM). The internalization assay was validated with an average of Z´=0.6+/- 0.10 for High Content Screening.

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