

Human Recombinant Muscarinic Acetylcholine Receptor M2 Stable Cell Line**Cat. No. M00258****Version 05292014**

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I. INTRODUCTION

Catalog Number: M00258

Cell Line: CHO-K1/M2/Gα15

Gene Synonyms: CHRM2

Expressed Gene: GenBank Accession Number NM_000739; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Applications: Functional assays for M2 receptor

Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Media: Ham's F12, 10% FBS, 200 µg/ml Zeocin, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Muscarinic acetylcholine receptors belong to a superfamily of seven-TM-domain receptors that interact with G-proteins to initiate intracellular responses. Five muscarinic receptor subtypes have been identified and named from M1 to M5.

The M2 muscarinic receptor couples to $G_{i/o}$ to inhibit cAMP production. GenScript co-transfected human M2 with Gα15 in the CHO-K1 which supports high levels of recombinant M2 expression on the cell surface and contains high levels of Gα15 to couple the receptor to the calcium signaling pathway.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritidis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

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III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Oxotremorine in CHO-K1/G α 15/M2 and CHO-K1/G α 15 cells

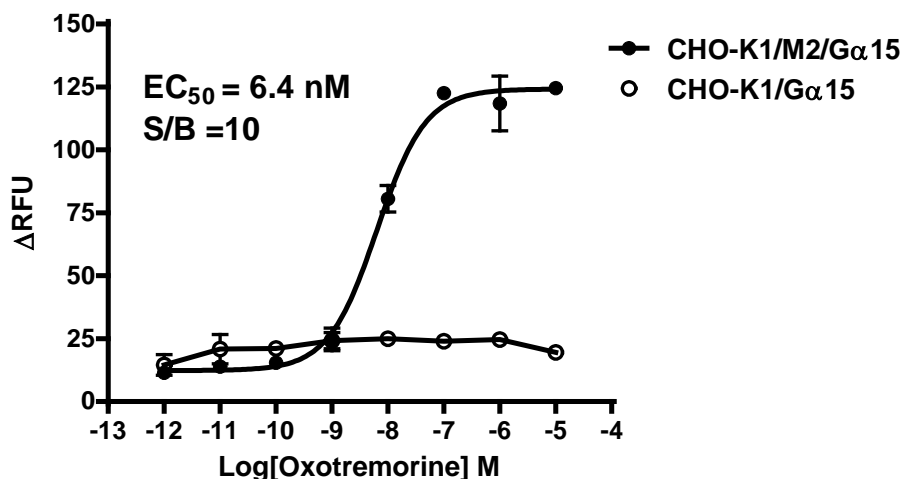


Figure 1. Oxotremorine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/G α 15/M2 and CHO-K1/G α 15 cells. The cells were loaded with Calcium-4 prior to stimulation with an M2 receptor agonist, Oxotremorine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Oxotremorine (Mean \pm SD, n = 2). The EC₅₀ of Oxotremorine on M2 in CHO-K1 cells was 6.4 nM. The S/B of Oxotremorine on M2 in CHO-K1 cells was 10.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$

X is the logarithm of concentration.
Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. RADIOLIGAND BINDING ASSAY

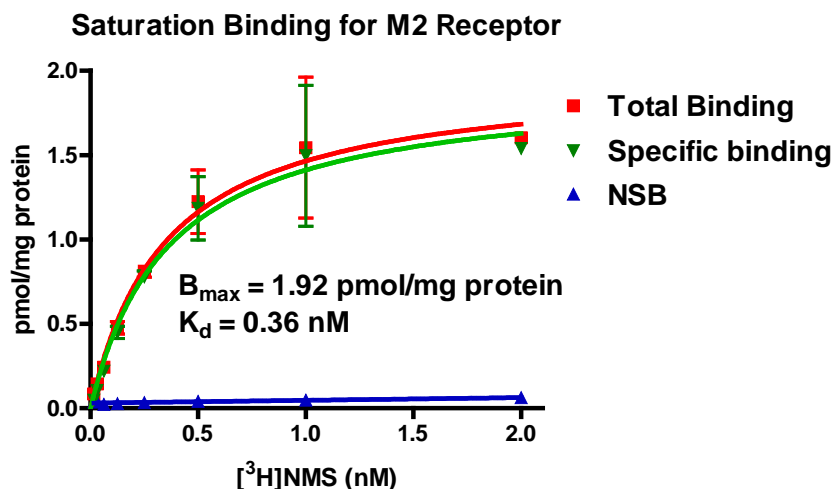


Figure 2. 10 μg of membranes prepared from CHO-K1 cells stably expressing M2 receptors were incubated with indicated concentrations of $[^3\text{H}]\text{N}$ -Methylscopolamine ($[^3\text{H}]\text{NMS}$) in the absence (total binding) or presence of 1000- fold excess unlabeled Atropine (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

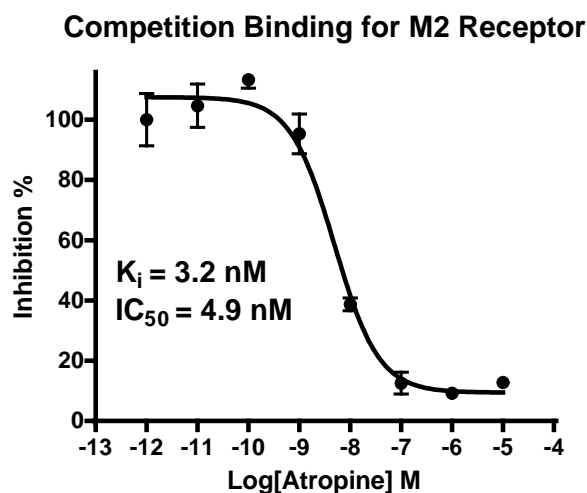


Figure 3. 10 μg of membranes prepared from CHO-K1 cells stably expressing M2 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM $[^3\text{H}]\text{N}$ -Methylscopolamine ($[^3\text{H}]\text{NMS}$). Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

V. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO₂.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

VI. REFERENCES

1. Goin JC, Nathanson NM. (2006) Quantitative analysis of muscarinic acetylcholine receptor homo- and heterodimerization in live cells: regulation of receptor down-regulation by heterodimerization. *J Biol Chem.*, 281(9):5416-25
2. Cembala TM *et al.* (1998) Interaction of neuromuscular blocking drugs with recombinant human m1-m5 muscarinic receptors expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.* 125(5):1088-94.
3. Mary H *et al.* (1995) Human muscarinic receptors expressed in A9L and CHO cells: activation by full and partial agonists. *Br J Pharmacol.* 114(6):1241-9.
4. Schwarz RD *et al.* (1993) Characterization of muscarinic agonists in recombinant cell lines. *Life Sci.* 52(5-6):465-72.

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