Human Recombinant Muscarinic Acetylcholine Receptor M1 Stable Cell Line

Cat. No. M00251 **Version 05292014**

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I. INTRODUCTION

Catalog Number: M00185
Cell Line Name: CHO-K1/M1
Gene Synonyms: M1; HM1; MGC30125; CHRM1
Expressed Gene: Genbank Accession Number NM_000738; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for M1 receptor
Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO
Complete Culture Medium: Ham's F12, 10% FBS
Culture Medium: Ham's F12, 10% FBS, 500 μg/ml Penicillin-Streptomycin, 200 μg/ml Zeocin
Mycoplasma Status: Negative
Storage: Liquid nitrogen upon receiving

II. BACKGROUND

M1 was expressed in the CNS such as cerebral cortex, basal ganglia, limbic areas, vestibular system and esophageal smooth muscle. Synaptic transmission by muscarinic acetylcholine receptors (mAChRs) is employed throughout the central and peripheral nervous systems to elicit a large and diverse array of neurophysiological actions. An important aspect of mAChR functional diversity is reflected by the multitude of biochemical and electrophysiological actions evoked by acetylcholine binding to mAChRs, which include the regulation of intracellular levels of cAMP, cGMP and inositol phospholipids, and the opening or closing of the potassium, calcium, and chloride ion channels found in certain tissues.

§: 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.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Carbachol in CHO-K1/M1 and CHO-K1 cells

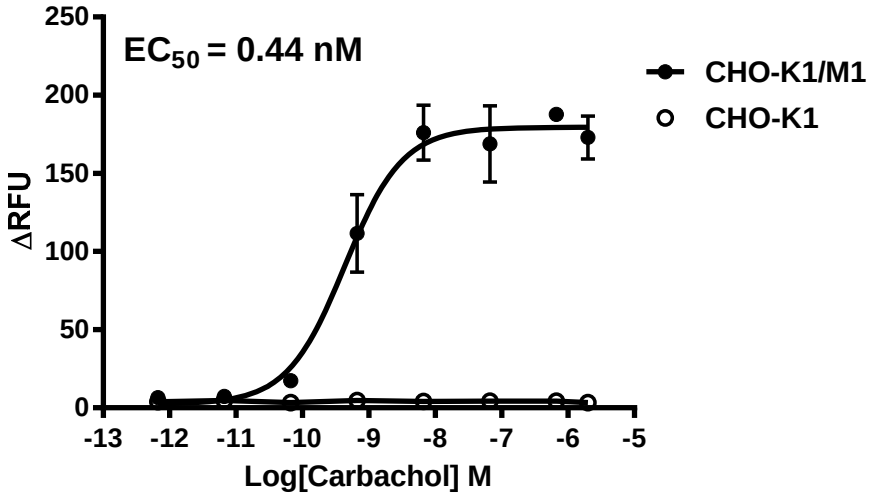

Figure 1. Carbachol-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an M1 receptor agonist, carbachol. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of carbachol (Mean ± SD, n = 2). The EC_{50} of carbachol on M1 in CHO-K1 cells was 0.44 nM. The S/B of carbachol on M1 in CHO-K1 cells was 76.

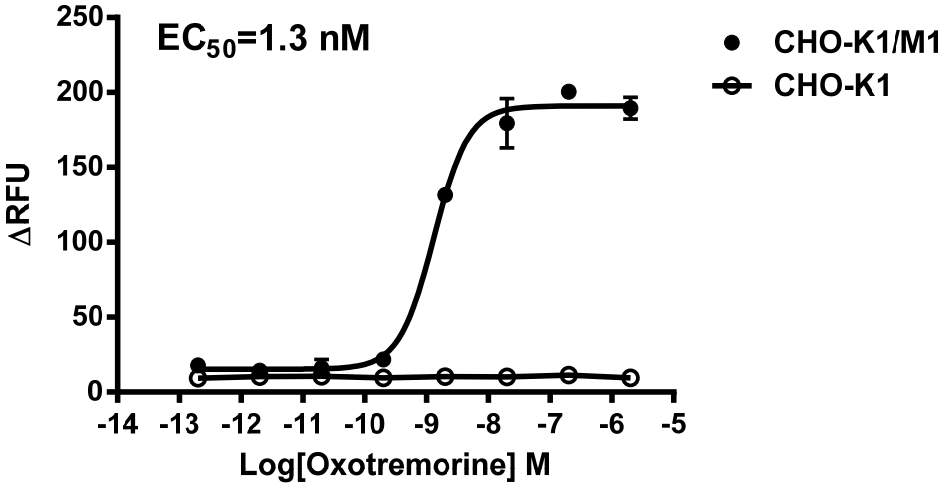

Figure 2. Oxotremorine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an M1 receptor agonist, oxotremorine. The intracellular calcium change was measured by FlexStation III. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of oxotremorine l (Mean ± SD, n = 2). The EC_{50} of

oxotremorine on M1 in CHO-K1 cells was 1.3nM. The S/B of oxotremorine on M1 in CHO-K1 cells was 15.5.

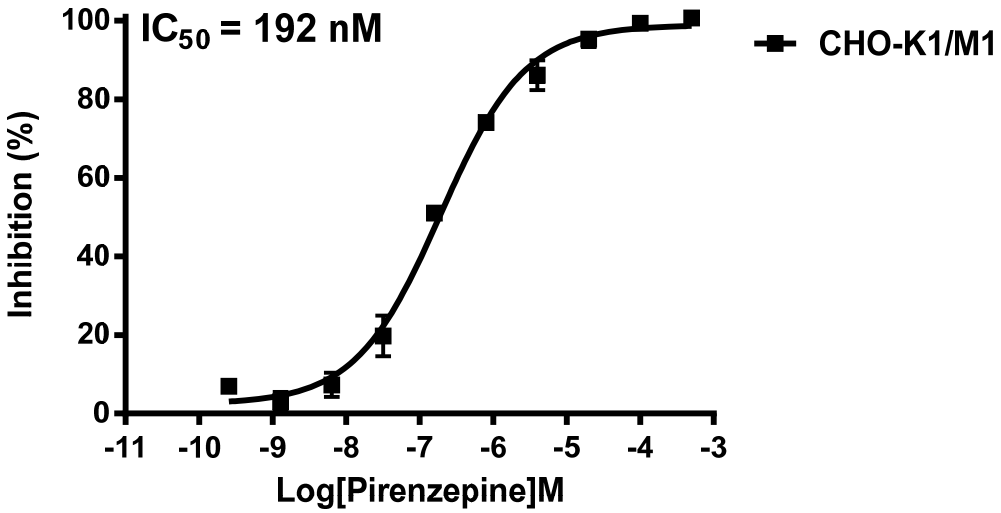

Figure 3. Pirenzepine-induced concentration-dependent inhibition of intracellular calcium mobilization in CHO-K1/M1 cells. The cells were loaded with Calcium-4 prior to inhibition with an M1 receptor antagonist, Pirenzepine then EC_{80} of agnosit oxotremorine. The intracellular calcium change was measured by FlexStation III. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Pirenzepine (Mean ± SD, n = 2). The IC_{50} of Pirenzepine on M1 in CHO-K1 cells was 192 nM.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*HillSlope))
   X is the logarithm of concentration.
   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to background Ratio (S/B) = Top/Bottom

IV. RADIOLIGAND BINDING ASSAY

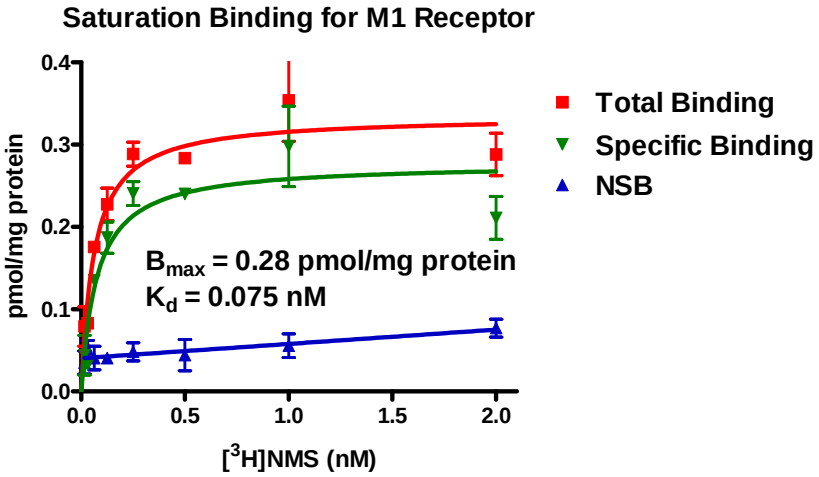

Figure 4. 10 µg of membranes prepared from CHO-K1 cells stably expressing M1 receptors were incubated with indicated concentrations of [^{3}H]N-Methylscopolamine ([^{3}H]NMS) in the absence (total binding) or presence of 1000-fold access unlabeled Atropine (nonspecific binding, NSB). Binding was terminated 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.

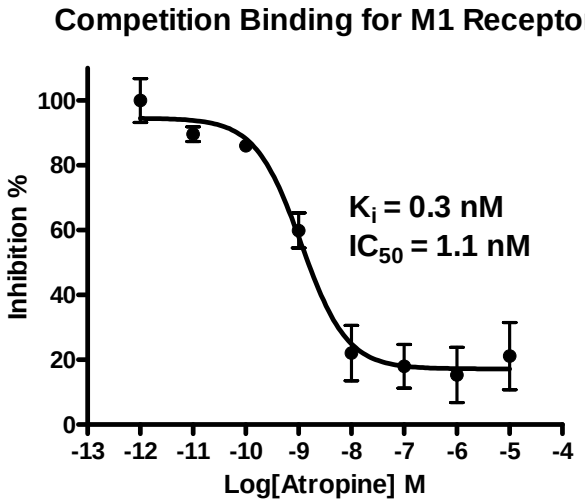

Figure 5. 10 µg of membranes prepared from CHO-K1 cells stably expressing M1 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM [^{3}H]N-Methylscopolamine ([^{3}H]NMS). Binding was terminated by rapid filtration. Data were fit to one-site competition equation 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 %CO_2.
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 %CO_2.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

VI. REFERENCES

1. Monica Lupu-Meiri (2001) Constitutive signaling by Kaposi's sarcoma-associated herpesvirus G-protein-coupled receptor desensitizes calcium mobilization by other receptors. *The Journal of Biological Chemistry.* Vol.276 No.10: 7122–7128.
2. Osamu Moro (1994) Overlapping multi-site domains of the muscarinic cholinergic Hm1 receptor involved in signal transduction and sequestration. *J. Biol. Chem*. Vol.269 No.9: 6651–6655
3. Lara M.Tolbert (1996) Human muscarinic cholinergic receptor Hm1 internalizes via clathrin-coated vesicles. *J. Biol. Chem.* Vol.271 No.29: 17335–17342
4. Petra Hogger (1995) Activating and inactivating mutations in N- and C-terminal i3 loop junctions of muscarinic acetylcholine Hm1 receptors. *J. Biol. Chem.* Vol.270. No.13: 7405–7410

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