

Human Recombinant Muscarinic Acetylcholine Receptor M1 Stable Cell Line

Technical Manual No. TM0386

Version 10132010

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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, 200 $\mu\text{g/ml}$ 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.

III. Representative Data

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

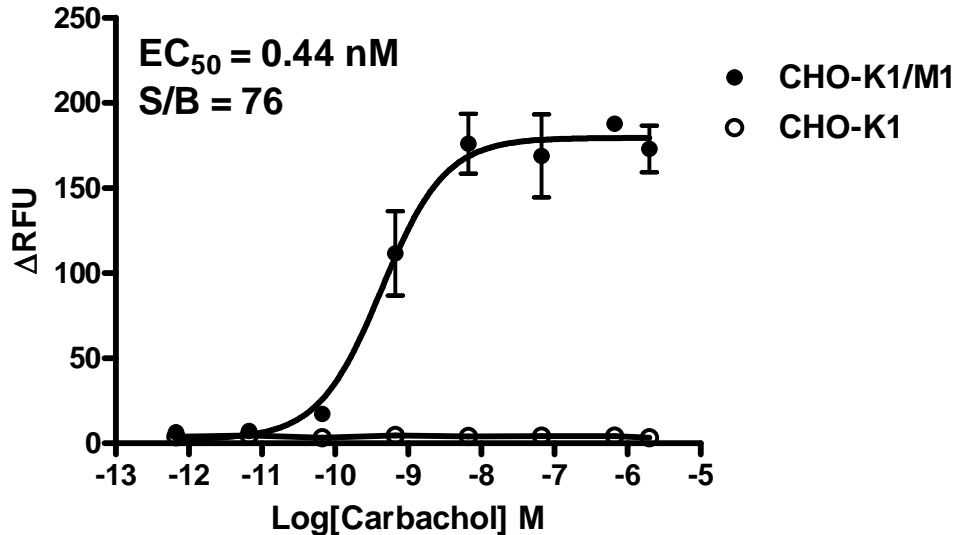


Figure 1. Carbachol-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an M1 receptor agonist, Carbachol. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Carbachol (Mean \pm SD, n = 2). The EC₅₀ 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.

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

Radioligand Binding Assay

Saturation Binding for M1 Receptor

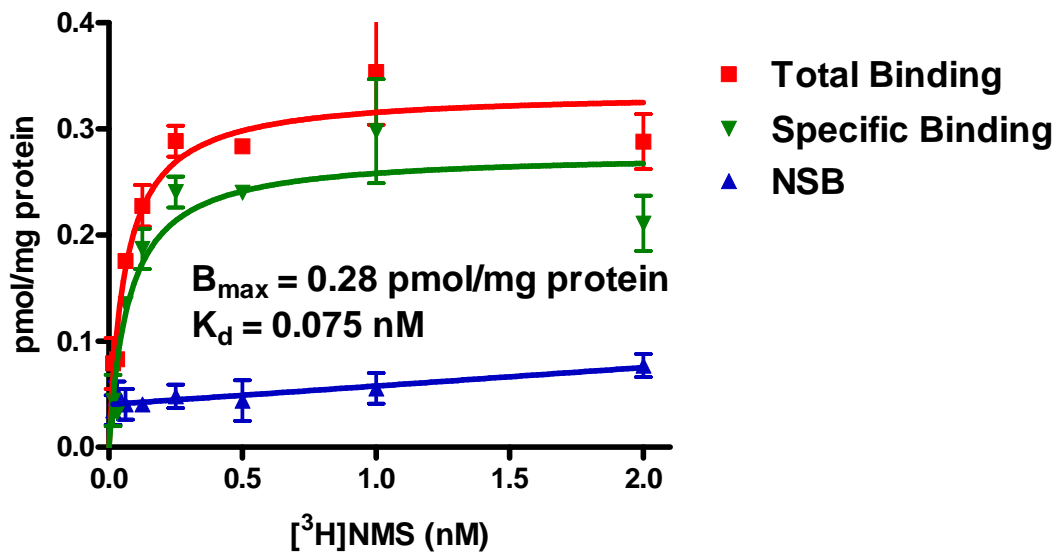


Figure 2. 10 µg of membranes prepared from CHO-K1 cells stably expressing M1 receptors were incubated with indicated concentrations of [³H]N-Methylscopolamine ([³H]NMS) in the absence (total binding) or presence of 1000-fold excess unlabeled Atropine (nonspecific binding, NSB). Binding was terminated 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.

Competition Binding for M1 Receptor

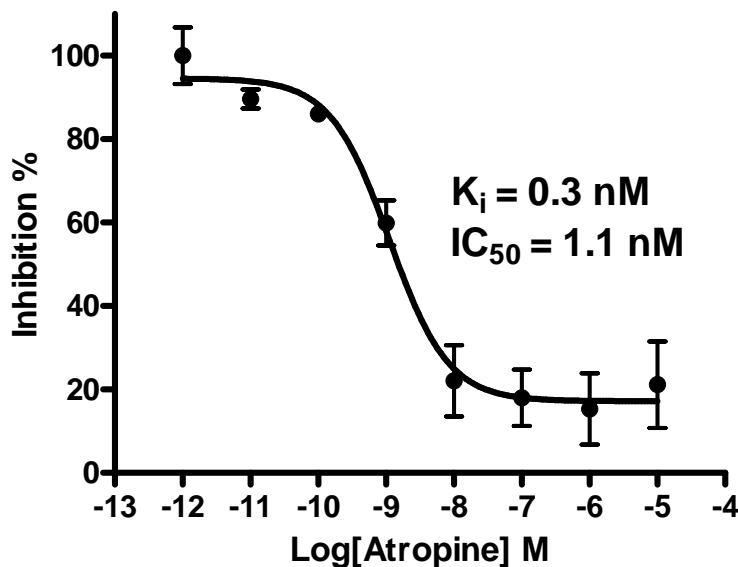


Figure 3. 10 µg of membranes prepared from CHO-K1 cells stably expressing M1 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM [³H]N-Methylscopolamine ([³H]NMS). Binding was terminated by rapid filtration. Data were fit to one-site competition equation using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

IV. 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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Zeocin to a concentration of 200 µg/ml the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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

Medium Renewal: Every 2 to 3 days

V. 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.
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3. Lara M.Tolbert (1996) Human muscarinic cholinergic receptor Hm1 internalizes via clathrin-coated vesicles. *J. Biol. Chem.* Vol.271 No.29: 17335–17342
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