Human Recombinant Muscarinic Acetylcholine Receptor M5 Stable Cell Line

Cat. No. M00186 **Version 05282014**

I. INTRODUCTION

Catalog No: M00186

Cell Line Name: CHO-K1/M5

Expressed Gene: Genbank Accession Number NM_012125

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for M5 receptor

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

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

Culture Medium: Ham's F12, 10% FBS, 200 μg/ml Zeocin

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, named M1 through M5. Receptors of the M5 receptor subtype couple through the $G_{q/11}$ class of G-proteins and activate the phospholipase C pathway. Activation of this pathway in turn leads to increases in free intracellular calcium levels as inositol triphosphate mediates release of calcium from the endoplasmic reticulum. RT-PCR reveals that M5 mRNA is quite uniformly expressed in brain. However, there is little data regarding the expression and function of the M5 receptor in peripheral tissues. Currently, it is clear that the M5 receptor, due to the high likelihood that its distribution is restricted to the CNS, probably plays a discrete role in dopaminergic transmission. Although the identification of M5 expression in salivary glands and iris-ciliary muscle suggests a broader role, the data on this is sparse and requires extensive confirmation.

§: 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/M5 and CHO-K1 cells

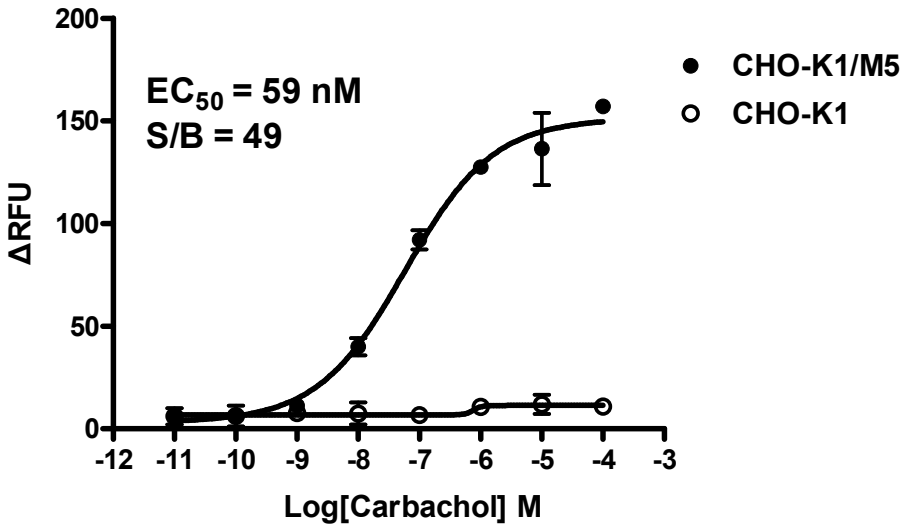

Figure 1. Carbachol-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M5 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an M5 receptor agonist, Carbachol. The intracellular calcium change was measured by FlexStation. The relative 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 M5 in CHO-K1 cells was 59 nM. The S/B of Carbachol on M1 in CHO-K1 cells was 49.

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

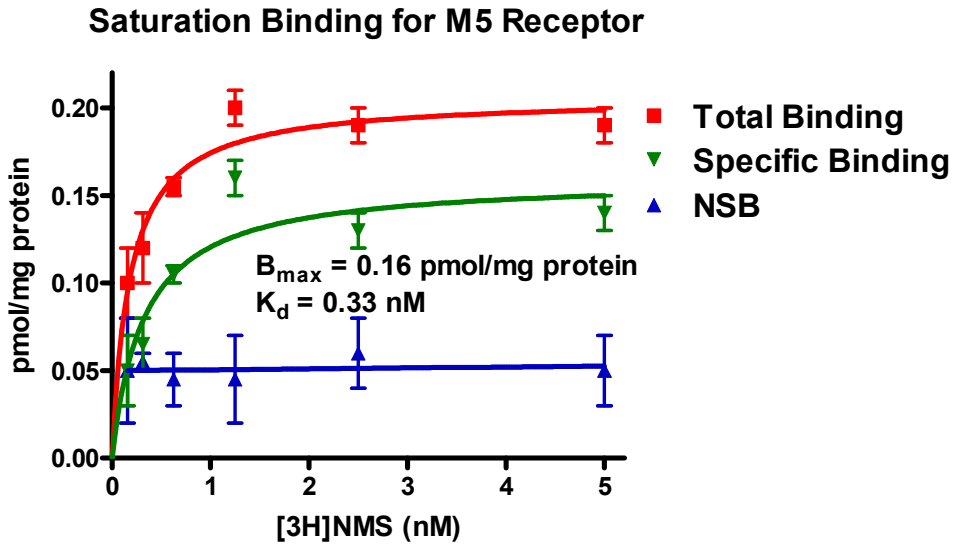

Figure 2. 10 µg of membranes prepared from CHO-K1 cells stably expressing M5 receptors were incubated with indicated concentrations of [^{3}H]N-Methylscopolamine ([^{3}H]NMS) in the absence (total binding) or presence of 1000-fold access unlabeled Atropine (nonspecific binding, NSB). Binding was terminated 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.

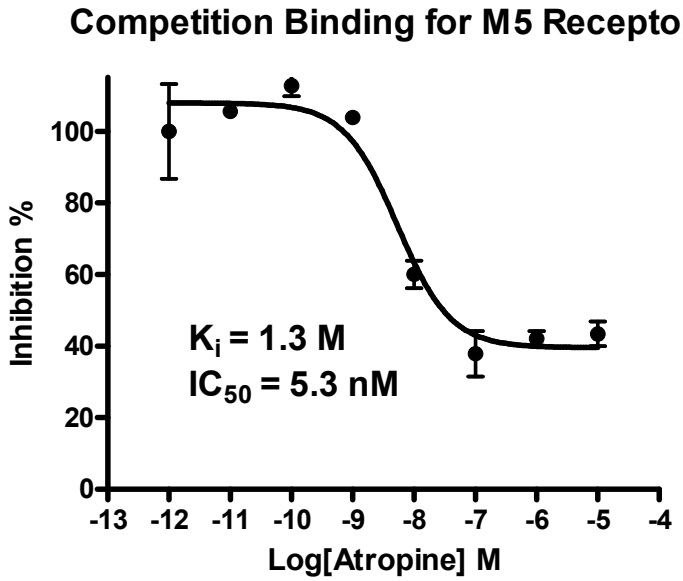

Figure 3. 10 µg of membranes prepared from CHO-K1 cells stably expressing M5 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM [^{3}H]N-Methylscopolamine ([^{3}H]NMS). Binding was terminated by rapid filtration. Data were fit to one-site competition equation 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.

Subculturing 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. Caulfield, M.P. (1993) Muscarinic receptors ± characterisation, coupling and function. *Pharmac Ther*, 58: 319 - 379.
2. Eglen, R.M. and Nahorski, S.R. (2000). The muscarinic M5 receptor: a silent or emerging subtype? *B. J. Pharmacology*, 130: 13 -21.
3. Wei, J., Walton, E.A., Milici, A., and Buafusco, J.J. (1994). M1 -M5 muscarinic receptor distribution in rat CNS by RT-PCR and HPLC. *J. Neurochem*, 63: 815 - 821.

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