Human Recombinant Muscarinic Acetylcholine Receptor M3 Stable Cell Line

Cat. No. M00259 **Version 05262014**

I. INTRODUCTION

Catalog Number: M00259
Cell Line: CHO-K1/M3
Expressed Gene: GenBank Accession Number NM_000740; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Applications: Functional assays for M3 receptor
Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO
Complete Growth Medium: Ham's F12, 10% FBS
Culture Medium: Ham's F12, 10% FBS, 400 μg/ml G418
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 M3 muscarinic receptors are located at many places in the body, e.g. smooth muscles, endocrine, exocrine glands, as well as lungs. They are also found in the CNS, where it induces emesis. They generally cause smooth muscle contraction and increased glandular secretions.

§: 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/M3 cells

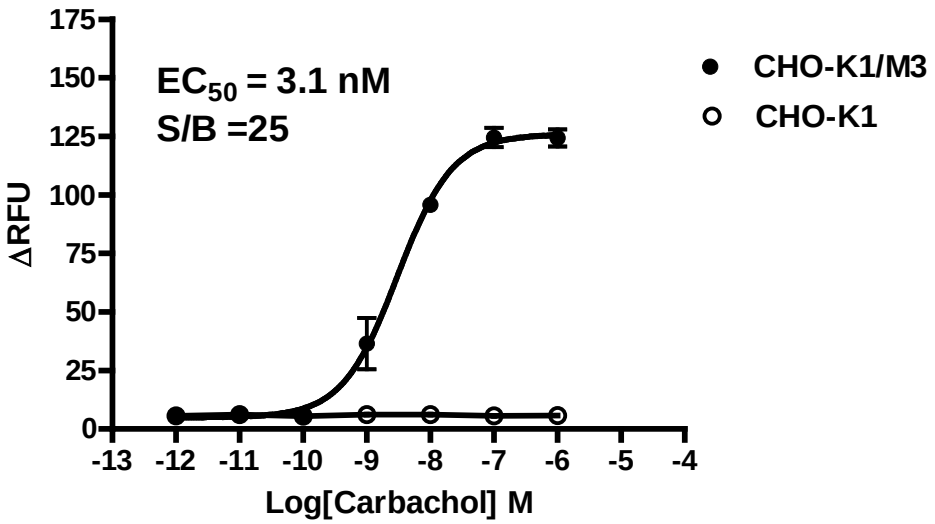

Figure 1. Carbachol-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M3 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an M3 receptor agonist, Carbachol. The intracellular calcium change was measured by FlexStation. The relative 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 M3 in CHO-K1 cells was 3.1 nM. The S/B of Carbachol on M3 in CHO-K1 cells was 25.

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

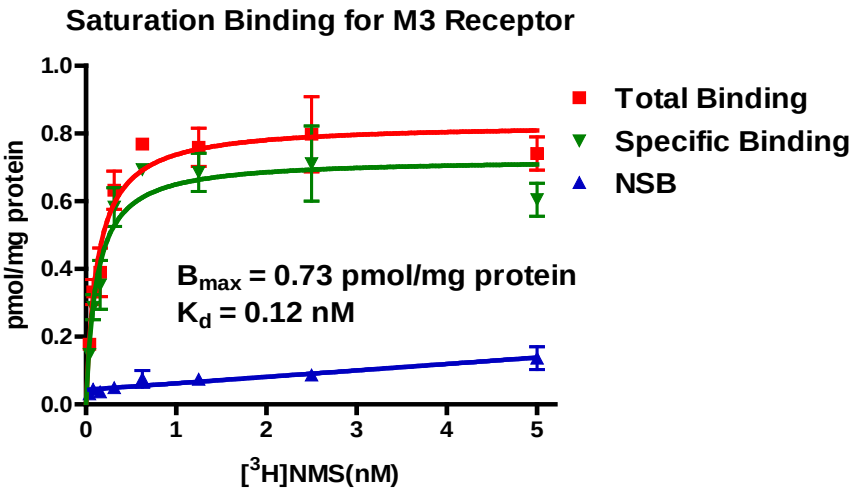

Figure 2 10 μg of membranes prepared from CHO-K1 cells stably expressing M3 receptors were incubated with indicated concentrations of [^{3}H]N-Methylscopolamine ([^{3}H]NMS) in the absence (total binding) or presence of 1000-fold access unlabeled Atropine (nonspecific binding, NSB). Binding was terminated 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.

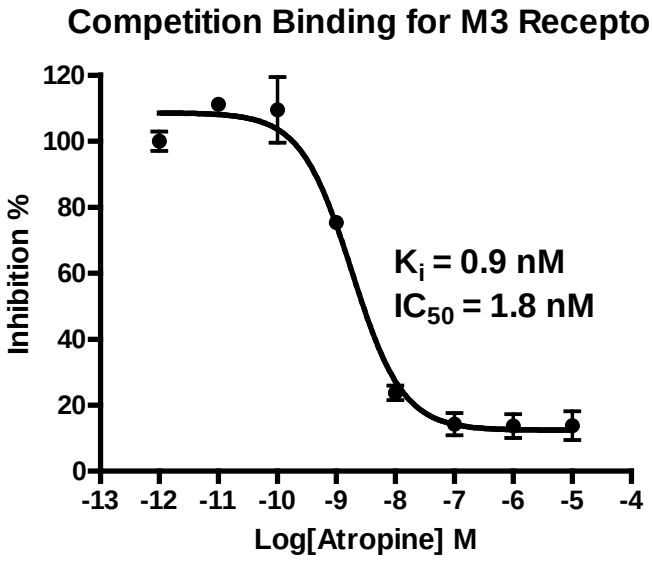

Figure 3 10 μg of membranes prepared from CHO-K1 cells stably expressing M3 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM [^{3}H]N-Methylscopolamine ([^{3}H]NMS). Binding was terminated by rapid filtration. Data were fit to one-site competition equation 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 %CO2.
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. 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*

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