Human Recombinant Muscarinic Acetylcholine Receptor M3 Stable Cell Line

Technical Manual No. TM0414 **Version 10132010**

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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.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Carbachol in CHO-K1/M5 and CHO-K1 cells

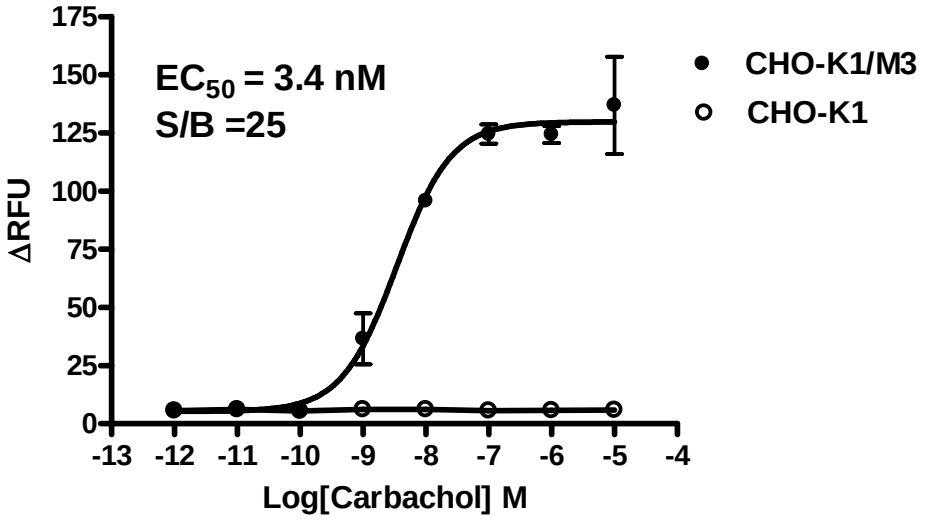

Figure 1. Carbachol-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M3 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an M3 receptor agonist, Carbachol. The intracellular calcium change was measured by FlexStation. The relative 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 3.4 nM. The S/B of Carbachol on M3 in CHO-K1 cells was 76.

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 the response

   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.

2. Signal to background Ratio (S/B) = Top/Bottom

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Radioligand Binding Assay

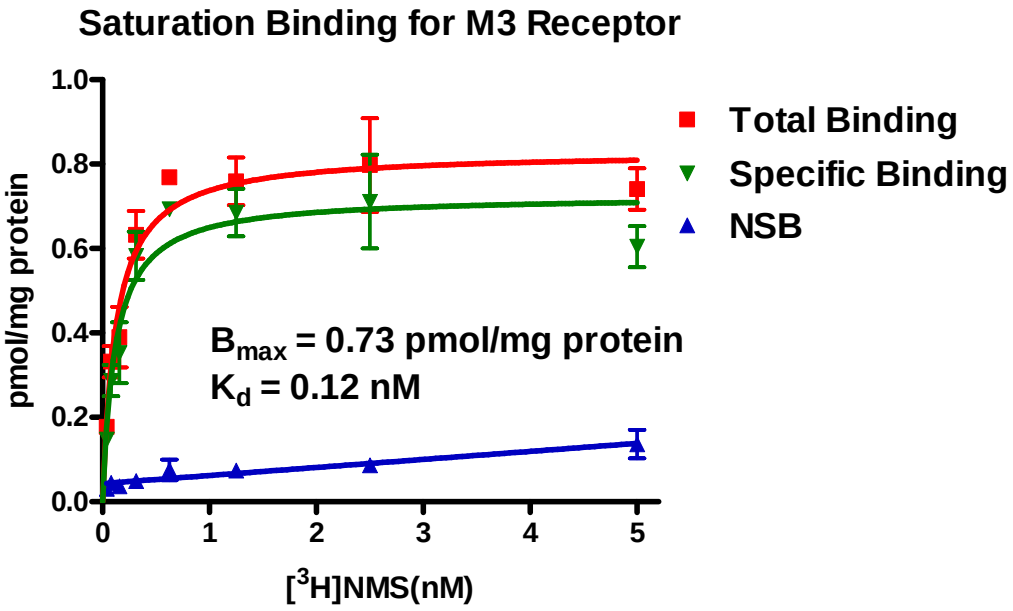

Figure 1 10 µg of membranes prepared from CHO-K1 cells stably expressing M3 receptors were incubated with indicated concentrations of [^{3}H]N-Methylscopolamine ([^{3}H]NMS) in the absence (total binding) or presence of 1000-fold access unlabeled Atropine (nonspecific binding, NSB). Binding was terminated 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.

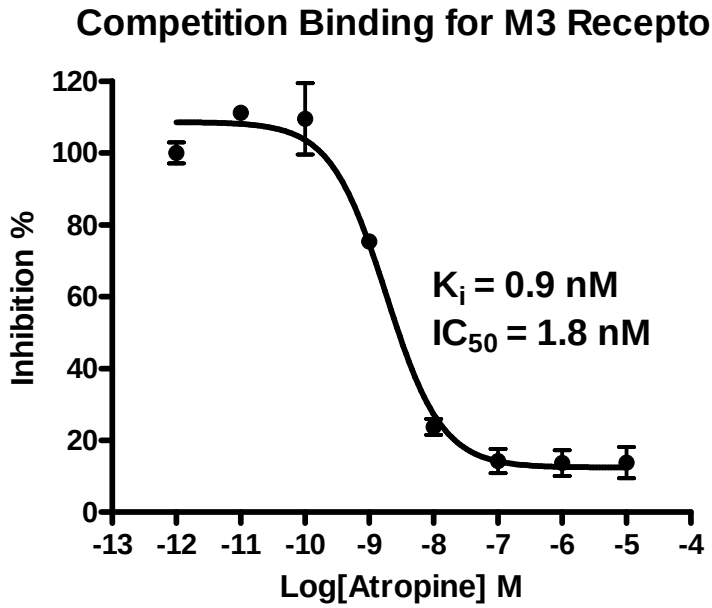

Figure 2 10 µg of membranes prepared from CHO-K1 cells stably expressing M3 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM [^{3}H]N-Methylscopolamine ([^{3}H]NMS). Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.

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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 G418 to a concentration of 400 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. 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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