GenScript

Make Research Easy

Human Recombinant Muscarinic Acetylcholine Receptor M4 Stable Cell Line

Cat. No. M00238 **Version 05282014**

I. INTRODUCTION

Catalog Number: M00238

Cell Line: CHO-K1/M4/Gα15

Gene Synonyms: CHRM4

Expressed Gene: GenBank Accession Number NM_000741; no expressed tags

Host Cell: CHO-K1/Gα15

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

Stability: 16 passages

Applications: Functional assays for M4 receptor

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

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

Culture Media: Ham's F12, 10% FBS, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B

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 M4 muscarinic receptor couples to $G_{i/o}$ to inhibit cAMP production. GenScript co-transfected human M4 with Gα15 in the CHO-K1 which supports high levels of recombinant M4 expression on the cell surface and contains high levels of Gα15 to couple the receptor to the calcium signaling pathway.

§: 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. EPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Oxotremorine in CHO-K1/Gα15/M4 and CHO-K1/Gα15 cells

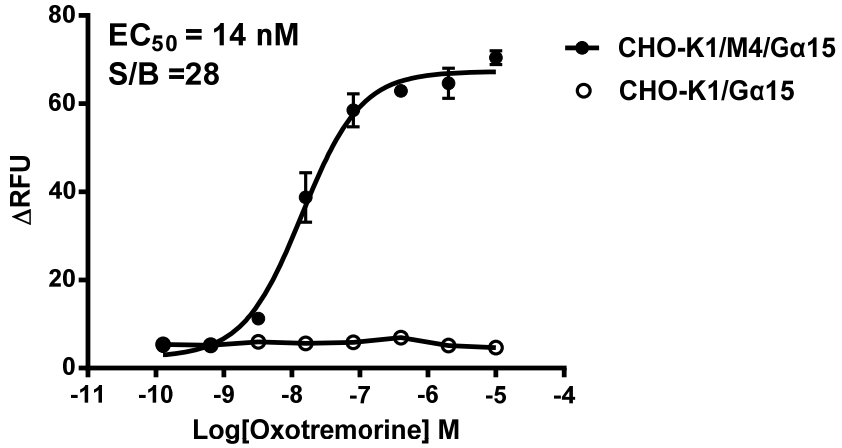

Figure 1. Oxotremorine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M4/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an M4 receptor agonist, Oxotremorine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Oxotremorine (Mean ± SD, n = 2). The EC_{50} of Oxotremorine on M4 co-expressing with Gα15 in CHO-K1 cells was 14 nM. The S/B of Oxotremorine on M4 co-expressing with Gα15 in CHO-K1 cells was 28.

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

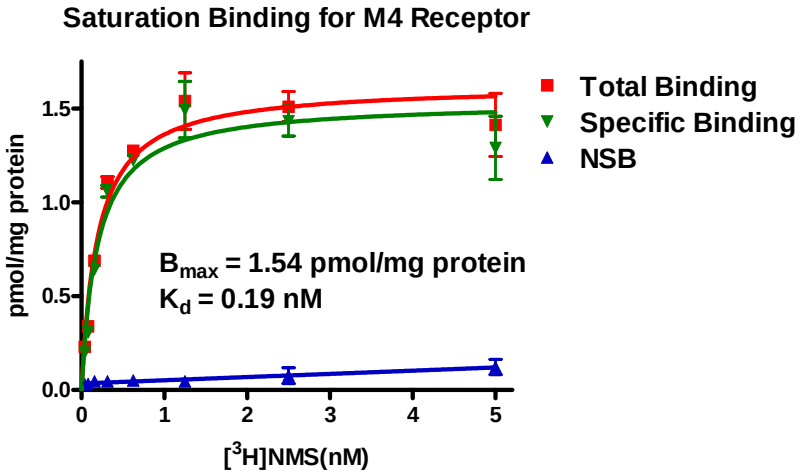

Figure 1 10 μg of membranes prepared from CHO-K1 cells stably expressing M4 receptors were incubated with indicated concentrations of [^{3}H]N-Methylscopolamine ([^{3}H]NMS) in the absence (total binding) or presence of 1000-fold access unlabeled Atropine (nonspecific binding, NSB). Binding was terminated 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.

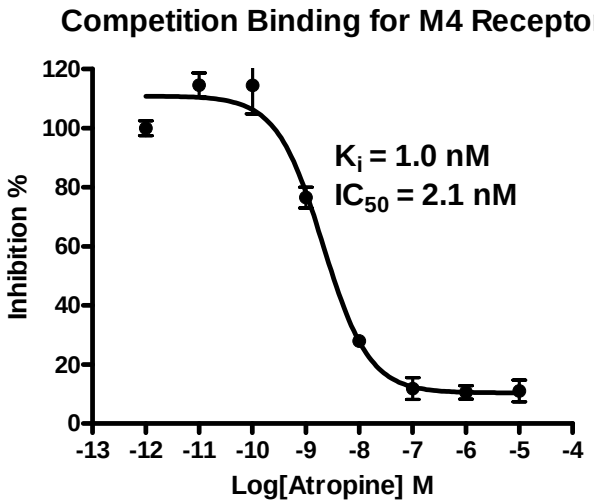

Figure 2 10 μg of membranes prepared from CHO-K1 cells stably expressing M4 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM [^{3}H]N-Methylscopolamine ([^{3}H]NMS). Binding was terminated by rapid filtration. Data were fit to one-site competition equation 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. 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*
2. Cembala TM *et al*. (1998) Interaction of neuromuscular blocking drugs with recombinant human m1-m5 muscarinic receptors expressed in Chinese hamster ovary cells. *Br. J. Pharmacol*. 125(5):1088-94.
3. Mary H *et al*. (1995) Human muscarinic receptors expressed in A9L and CHO cells: activation by full and partial agonists. *Br J Pharmacol.* 114(6):1241-9.
4. Schwarz RD *et al*. (1993) Characterization of muscarinic agonists in recombinant cell lines. *Life Sci.* 52(5-6):465-72.

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