

Human Recombinant Muscarinic Acetylcholine Receptor M5 Stable Cell Line

Technical Manual No. TM0373

Version 10132010

I	Introduction	1
II	Background.....	1
III	Representative Data.....	2
IV	Thawing and Subculturing.....	3
V	References	4
	Limited Use License Agreement.....	5

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 μ g/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.

III. Representative Data

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

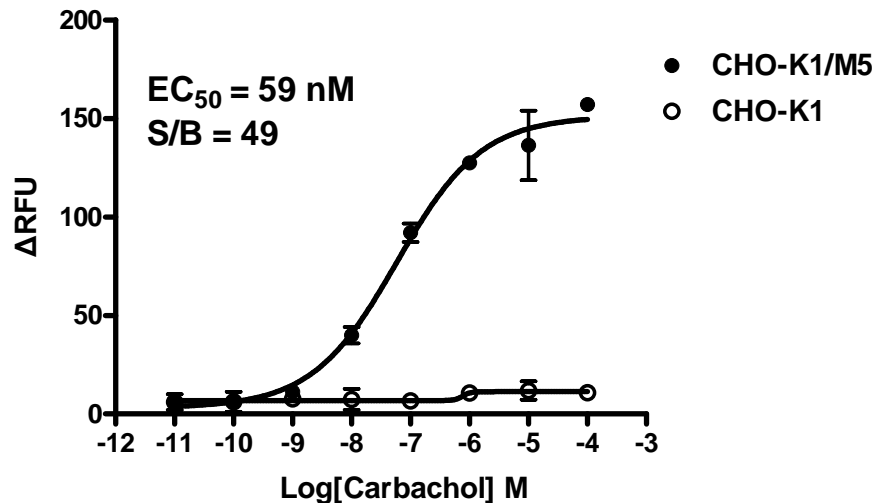


Figure 1. Carbachol-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/M5 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an M5 receptor agonist, Carbachol. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Carbachol (Mean \pm 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:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{Log}EC_{50} - X) * \text{HillSlope}))}$$

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

Radioligand Binding Assay

Saturation Binding for M5 Receptor

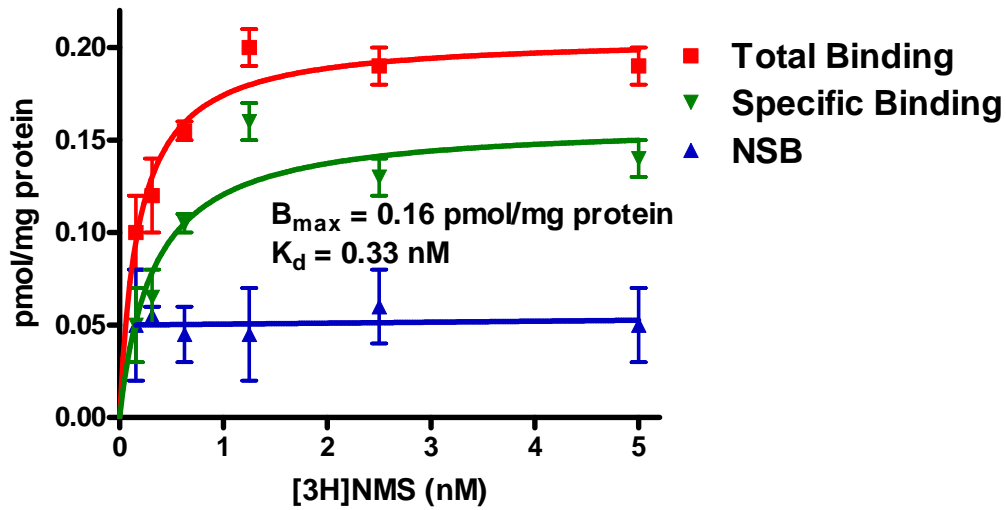


Figure 2. 10 μ g of membranes prepared from CHO-K1 cells stably expressing M5 receptors were incubated with indicated concentrations of [³H]N-Methylscopolamine ([³H]NMS) in the absence (total binding) or presence of 1000-fold excess unlabeled Atropine (nonspecific binding, NSB). Binding was terminated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

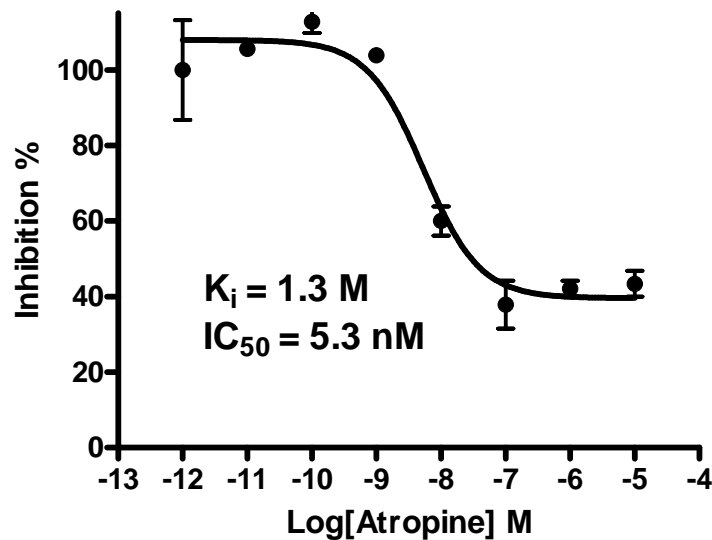


Figure 3. 10 μ g of membranes prepared from CHO-K1 cells stably expressing M5 receptors were incubated with indicated concentrations of Atropine in the presence of 0.2 nM [³H]N-Methylscopolamine ([³H]NMS). Binding was terminated by rapid filtration. Data were fit to one-site competition equation using 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. Caulfield, M.P. (1993) Muscarinic receptors \pm 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.

GenScript USA Inc.
860 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: <http://www.genscript.com>

For Research Use Only.

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

- 1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
- 2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
- 3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 860 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.