CHO-K1/ M5 Cells Ready-to-Use™

Technical Manual No. TM0372 **Version 02192009**

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I. PRODUCT INFORMATION

Catalog No: M00175 Lot.No: D206901

Host Cell: CHO-K1 Expressed Gene: Genbank Accession Number: NM_012125

Quantity: 1×10^7/vial Mycoplasma Status: Negative

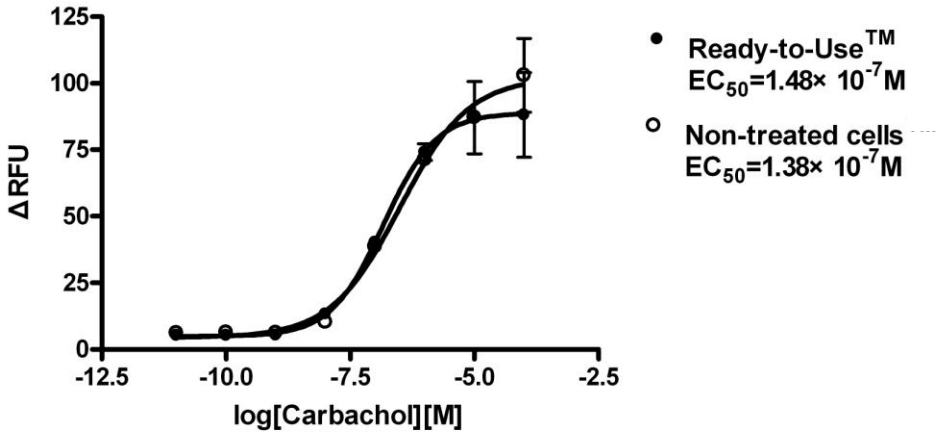
Storage: Liquid nitrogen Freeze Medium: Culture medium plus 10% (V/V) DMSO

Culture Medium: F12, 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 250 μg/ml Zeocin

II. BACKGROUND

The muscarinic acetylcholine receptors belong to the super-family 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 Gq/11 class of G-proteins and activate the phopholipase 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



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This diagram depicts the calcium dose-responses of carbachol stimulation of CHO-K1/M5 Frozen Cells Ready-to-Use™ (●) and non-treated cells (○). The resulting increase in intracellular calcium was measured on a FlexStation fluorescent plate reader. Data points represent the average +/- standard deviation of triplicate determinations.

IV. ASSAY PROCEDURE

Calcium flux assay of CHO-K1/M5 receptor cell with FLIPRP® Calcium 4 Assay Kit:

1. Prepare carbachol addition plates in advance.
2. Seed cells at a density of 2×10^4cells/well in a 384-well black plate.
3. Incubate the cells overnight at 37°C and 5% CO_2.
4. Remove the cell plates from the incubator and add an equal volume of calcium4 loading buffer to each well (for a 384-well plate, this will be 25μL per well).
5. Incubate the plate at 37°C in the dark for one hour and then at room temperature for half an hour.
6. Add 5X working concentration of carbachol in HEPES buffered HBSS solution (PH=7.4) to cell plate. For a 384-well plate, this will be 12.5 μl/well.
7. Read with FlexStation III, FLIPR, or a similar fluorescence plate reader. The signal should be measured for two minutes. Add carbachol 20 seconds into the measurement.

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