

Product Information

M₂ MUSCARINIC RECEPTOR, HUMAN RECOMBINANT (CHO)

Product Number **M 4560**

Product Description

Acetylcholine receptors are made of two very different families of receptors, the muscarinic acetylcholine receptors and the nicotinic acetylcholine receptors. The muscarinic receptors are members of the superfamily of G protein-coupled receptors while the nicotinic receptors are ligand gated ion channels. They were originally characterized based on their sensitivity to the agonists muscarine and nicotine.

Muscarinic acetylcholine receptors (mAChR) are relatively abundant and mediate the diverse actions of acetylcholine in the CNS as well as in non-nervous tissues innervated by the parasympathetic nervous system. There are five subtypes of muscarinic receptors encoded by five separate genes (M₁-M₅). Their transmembrane spanning domains show strong sequence homology with each other and with related G-protein-coupled receptors (GPCRs), but each receptor also has a unique amino terminal (extracellular), and third intracellular loop (I3). As with the biogenic amine receptors, much of the effector coupling specificity of these receptors resides in their intracellular domains, in particular the third loop.

In nervous tissues, M₂ muscarinic receptors appear to frequently inhibit neuronal firing may also be found on axon terminals where they inhibit neurotransmitter release (autoreceptors or heteroreceptors). The M₂ muscarinic receptor inhibits adenylyl cyclase in smooth muscle; as a consequence the oppose the effects of adrenergic innervation. In cardiac tissue, M₂ muscarinic receptors activate G protein-coupled potassium channels to hyperpolarize the muscle, contributing to the slowing of the heart rate.

Reagents

M₂ Muscarinic Receptor, Human Recombinant (CHO) is provided as purified membranes suspended in 50 mM Tris-HCl at pH 7.4 containing 10% glycerol and 1% bovine serum albumin.

Storage/Stability

Store tightly sealed at -70 °C. When stored in its original packaging solution, the membranes retain their original specific activity for several months.

Procedure

Incubation buffer

50 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 mM EDTA

Binding Protocol

1. Membranes
Dilute in incubation buffer (0.5 ml of membranes to 24.5 ml of incubation buffer).
2. Assay mixture
500 µl of diluted membranes
20 µl of [³H]-radioligand in buffer
20 µl of incubation buffer or unlabeled ligand in buffer
540 µl of incubation buffer
Radioligand: [³H]-N-Methyl-scopolamine at a final concentration of 0.2 nM for competition studies.
Unlabeled ligand: Atropine (Cat. No. A-105) at a final concentration of 1 µM.
3. Incubation time
60 minutes at 27°C
4. Separation
Over GF/C filters (presoaked in 0.3% polyethylenimine, Cat. No. P-182) then wash 9x with ice cold 50 mM Tris-HCl at pH 7.4 at 4 °C.

Results

Typical affinities using standard binding assay above. Results may vary from lot to lot.

Ligand	Affinity (K _i) (nM)
Methoctramine (M-105)	20
<i>p</i> -F-Hexahydro-sila-difenidol (H-127)	1100
Pirenzepine (P-114)	1500

References

1. Gurevich, V. V., et al., "Binding wild type and chimeric arrestins to the M₂ muscarinic cholinergic receptor." J. Biol. Chem. **268**, 16879-16882 (1993).
2. Pepitoni, S., et al., "Phospholipase D activity and phosphatidylethanol formation in stimulated HeLa cells expressing the human M₁ muscarinic acetylcholine receptor gene." Biochem. Biophys. Res. Commun. **176**, 453-458 (1991).

JWM/PSS 01/03

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.