

M4 MUSCARINIC RECEPTOR, HUMAN (CHO)Product Number **M 4810****Product Description**

Muscarinic acetylcholine receptors are members of the superfamily of G protein-coupled receptors, abundant in the central nervous system (CNS) and innervated tissues.¹

The striatum is the main localization site of M4 muscarinic receptors. They are located on half of all projection neurons, and their mRNA is prevalent only in striatonigral neurons.² The M4 muscarinic receptor subtype specifically regulates dopamine D₁ receptor stimulated locomotor activity and mediates the negative feedback mechanism of acetylcholine secretion from postganglionic cholinergic nerve terminals.³

Reagent

M4 Muscarinic Receptor, human recombinant (CHO) is a frozen aliquot of membranes from CHO cells transfected with the human recombinant M4 muscarinic receptor. It is supplied in 50 mM TRIS-HCl, pH 7.4 containing 10% glycerol and 1% bovine serum albumin (BSA).

Procedure*Incubation buffer.*

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

*Binding Protocol***Membranes:**

Dilute in incubation buffer (0.5 ml of membrane + 24.5 ml incubation buffer).

Assay mixture:

500 µl diluted membranes
20 µl radioligand (Scopolamine at 0.2 nM for competition studies)
20 µl buffer or unlabeled ligand (Atropine at a concentration 1 µM)

Incubation time:

60 minutes at 27 °C

Product Information**Separation:**

Over GF/C filter (5 mm diameter, presoaked in 0.3% polyethylamine prepared in incubation buffer) then washed 4x with 500 µl of ice cold 50 mM TRIS-HCl, pH 7.4 at 4 °C.

Results

Typical data obtained with M4 muscarinic receptors. Specific values may vary from lot to lot.

Affinity Constants for selected antagonists

Ligand	K ₁ (nM)
[³ H]N-Methyl Scopolamine	0.2 (Kd)
4-DAMP-Methiodide	2.5
p-F-Hexahydro-sila-difenidol	50
Pirenzepine	65
Methoctramine	112

Affinity Constants for selected agonists

Ligand	K _H (µM)	K _L (µM)	High affinity sites (%)
Carbachol	0.8	81	61
Oxotremorine-M	0.04	5.0	62

Storage/Stability

Store tightly sealed at -70 °C. Long-term stability of this product is being evaluated. Initial studies indicate that the receptor can retain its original specific activity for several months when stored at -70 °C in its original packing solution. Repeated freeze-thaw of this product is not recommended.

Precautions

While no human toxicity data is available for this substance, it should be handled with care. Precautions should be taken to avoid contact by all routes of exposure.

References

1. Gomeza, J., et al., Life Sci., **68**, 2457-2466 (2001).
2. Santiago, M.P. and Potter, L.T., Brain Res., **894**, 12-20 (2001).
3. Gomeza, J., et al., Proc. Natl. Acad. Sci. USA, **96**, 10483-10488 (1999).

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