

human Muscarinic M₄ Receptor

Product No.: RBHM4M400UA

Lot No.: 2978704

Material Provided

Membranes: 1 x 400 units / 1000 µL frozen aliquot

Product Information

Cellular Background: CHO-K1

GenBank Accession Number: NM_000741

Unit Size: 24 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl₂, 10% sucrose.

Storage Conditions: Store at -80°C. Freeze-thaw is not recommended as it can affect product performance and homogeneity. In order to minimize negative impact of freeze-thawing, flash freeze in liquid nitrogen for 30 seconds prior to transferring to -80°C.

Stability: This product is stable for at least 3 years from reception if used and stored under recommended conditions.

Quality Control

B_{max} and K_d are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method ⁽¹⁾. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B_{max} in fmoles) by the maximal signal of a pre-defined reference tested in parallel. RTR is an indicator of lot-to-lot consistency. *We certify that these results meet our quality release criteria.

Ratio-to-Reference (RTR): 0.7

Expression Level (B_{max}): 2.4 pmol/mg membrane protein.

K_d for [³H]-Scopolamine (N-methyl): 0.05 nM

Protein Concentration: 9.6 µg/µL

(1) Smith, P.K., et al. (1985). *Anal. Biochem.* 150, 76-85.

Recommended Assay Conditions

| | |
|------------------------|---|
| Assay Buffer: | PBS pH 7.4 |
| Wash Buffer: | 50 mM Tris-HCl pH 7.4, 154 mM NaCl |
| Binding Protocol: | Binding assays are performed in 550 μ L total volume according to the following conditions: |
| 1 - Membrane dilution: | 0.125 mL of membranes + 24.875 mL assay buffer (1:200 dilution) |
| 2 - Incubation: | 25 μ L of incubation buffer or Atropine (Sigma A025) 5 μ M final for non specific binding (Saturation binding assay) |
| | <i>For competition binding assay: 25 μL of reference compounds at decreasing concentrations (see figure 2)</i> |
| | 25 μ L of radioligand at the appropriate concentration (see graph below) 500 μ L of diluted membranes |
| 3 - Incubation time: | 120 minutes at 27 $^{\circ}$ C |
| 4 - Filtration: | aspirate and wash 9 x 500 μ L with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI). |

Lot Specific Data

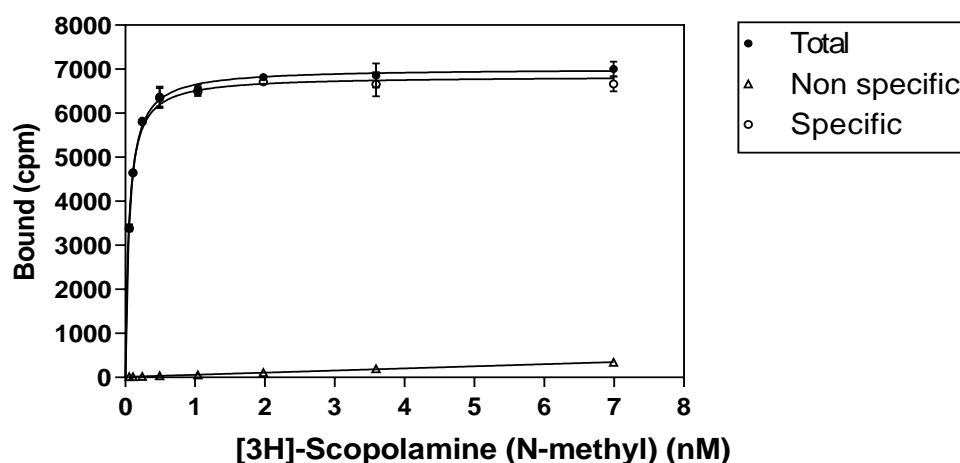


Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (24 μ g membranes/well, TopCount[®]) using [³H]-Scopolamine (N-methyl) (Revvity NET636 Lot No.: 2970776)

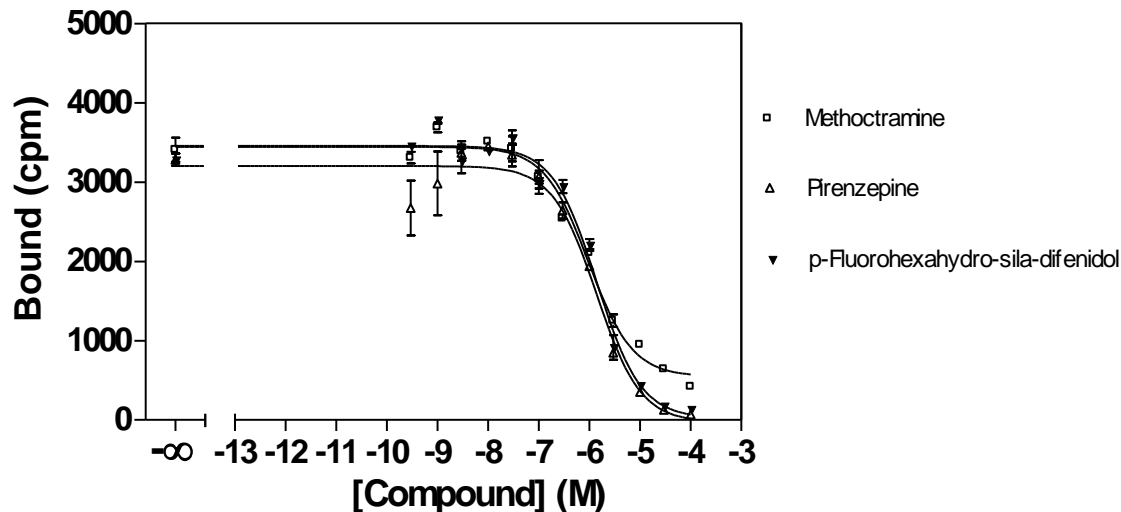


Figure 2: Competition binding assay curve (filtration)
 96-well competition binding assay curve (24 μ g membranes/well, TopCount®). Recommended radioligand concentration = 0.4 nM.

*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

| Reference Compounds | Ki (nM) |
|----------------------------------|---------|
| Methoctramine | 147 |
| Pirenzepine | 214 |
| p-Fluorohexahydro-sila-difenidol | 216 |
| | |

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