

## human Muscarinic M<sub>5</sub> Receptor

Product No.: RBHM5M400UA

Lot No.: 2110651

### Material Provided

Membranes: 1 x 400 units / 1000 µL frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: NM\_012125

Unit Size: 16 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl<sub>2</sub>, 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<sub>max</sub> and K<sub>d</sub> are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method <sup>(1)</sup>. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B<sub>max</sub> 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): 1.2

Expression Level (B<sub>max</sub>): 7.6 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>3</sup>H]-Scopolamine (N-methyl): 0.16 nM

Protein Concentration: 6.4 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 $\mu$ 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 $\mu$ L of incubation buffer or Atropine (Sigma A025) 5 $\mu$ M final for non specific binding (Saturation binding assay)
	<i>For competition binding assay: 25 <math>\mu</math>L of reference compounds at decreasing concentrations (see figure 2)</i>
	25 $\mu$ L of radioligand at the appropriate concentration (see graph below)
	500 $\mu$ L of diluted membranes
3 - Incubation time:	120 minutes at 27 $^{\circ}$ C
4 - Filtration:	aspirate and wash 9 x 500 $\mu$ L with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

## Lot Specific Data

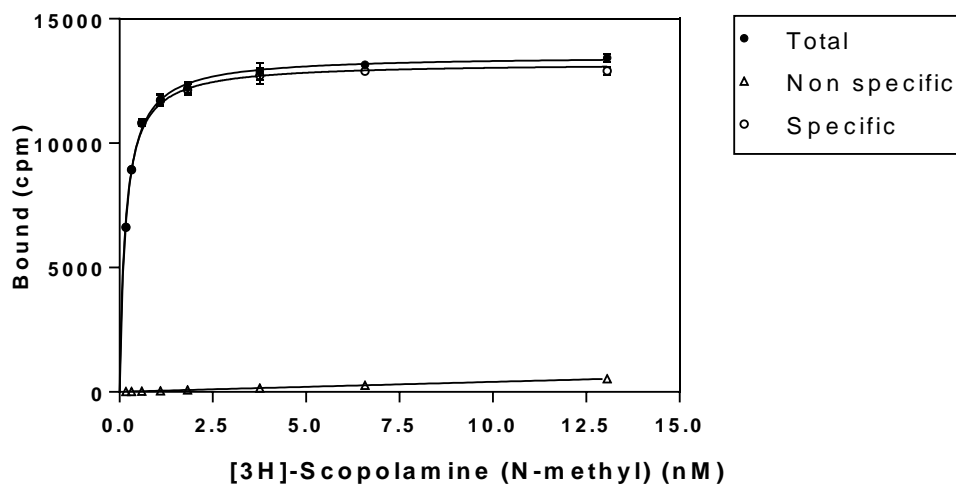


Figure 1: Saturation binding assay curve (filtration)  
 96-well saturation binding assay curve (16  $\mu$ g membranes/well, TopCount<sup>®</sup>) using [<sup>3</sup>H]-Scopolamine (N-methyl) (Revvity NET636 Lot No.: 2032889)

Typical Product Data

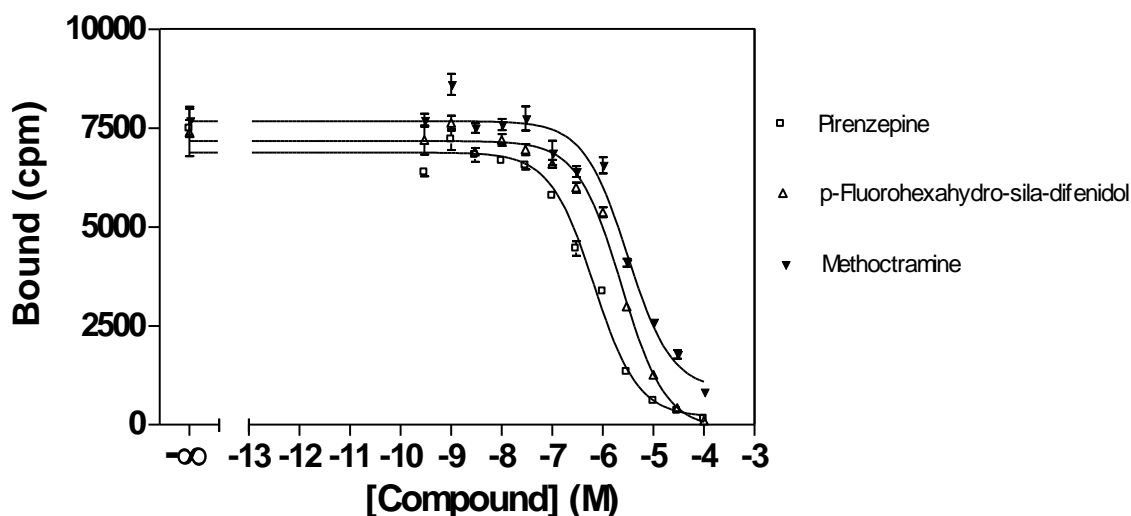


Figure 2: Competition binding assay curve (filtration)  
 96-well competition binding assay curve (16 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.4 nM.

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

Reference Compounds	Ki (nM)
Pirenzepine	361
p-Fluorohexahydro-sila-difenidol	1258
Methoctramine	1672

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