

Caution: For Laboratory Use. A research reagent for research purposes only

## human Muscarinic M<sub>1</sub> Receptor

**Product No.: RBHM1M400UA**

**Lot No.: 1815281**

### Material Provided

**Membranes:** 1 x 400 units / 1000 µL frozen aliquot

### Product Information

**Cellular Background:** CHO-K1

**GenBank Accession Number:** NM\_000738

**Unit Size:** 35 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):** 1.3

**Expression Level (B<sub>MAX</sub>):** 6.32 pmol/mg membrane protein.

**K<sub>D</sub> for [<sup>3</sup>H]-Scopolamine (N-methyl) :** 0.2 nM

**Protein Concentration:** 14 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)

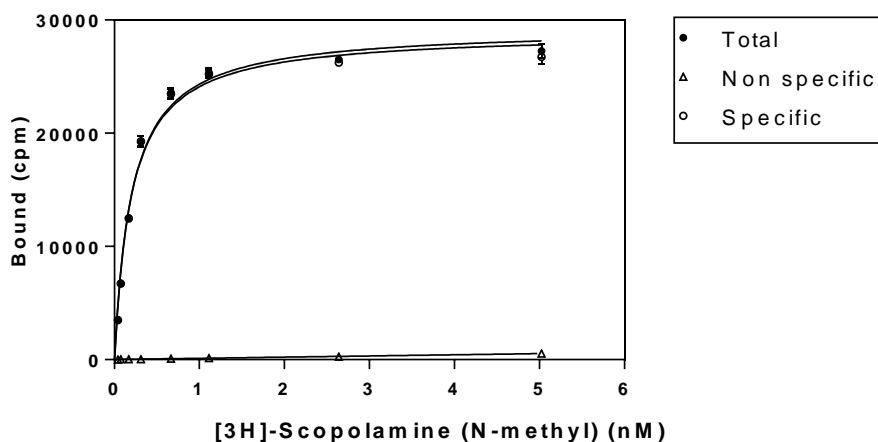
*For competition binding assay: 25  $\mu$ L of reference compounds at decreasing concentrations (see figure 2)*

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).

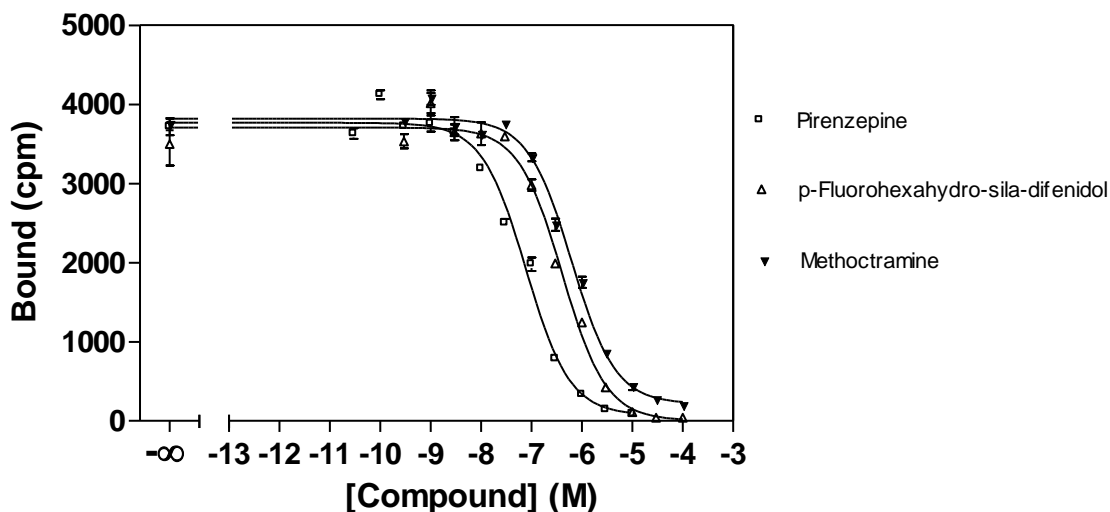
## Lot Specific Data



**Figure 1: Saturation binding assay curve (filtration)**

96-well saturation binding assay curve (35  $\mu$ g membranes/well, TopCount $^{\circ}$ ) using [ $^3$ H]-Scopolamine (N-methyl) (PerkinElmer NET636 Lot No.: 1770693)

## Typical Product Data



**Figure 2: Competition binding assay curve (filtration)**

96-well competition binding assay curve (35  $\mu$ g membranes/well, TopCount®). Recommended radioligand concentration = 0.2 nM.

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

Reference Compounds	K <sub>i</sub> (nM)
Pirenzepine	26
p-Fluorohexahydro-sila-difenidol	138
Methoctramine	213

## Suggested Materials and Instrumentation

Please visit our website

[www.perkinelmer.com/GPCR](http://www.perkinelmer.com/GPCR)

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