

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

human Muscarinic M₁ Receptor

Product No.: RBHM1M400UA

Lot No.: 1777380

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 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): 1.33

Expression Level (B_{MAX}): 6.332 pmol/mg membrane protein.

K_D for [³H]-Scopolamine (N-methyl): 0.16 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 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)

For competition binding assay: 25 μ L of reference compounds at decreasing concentrations (see figure 2)

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

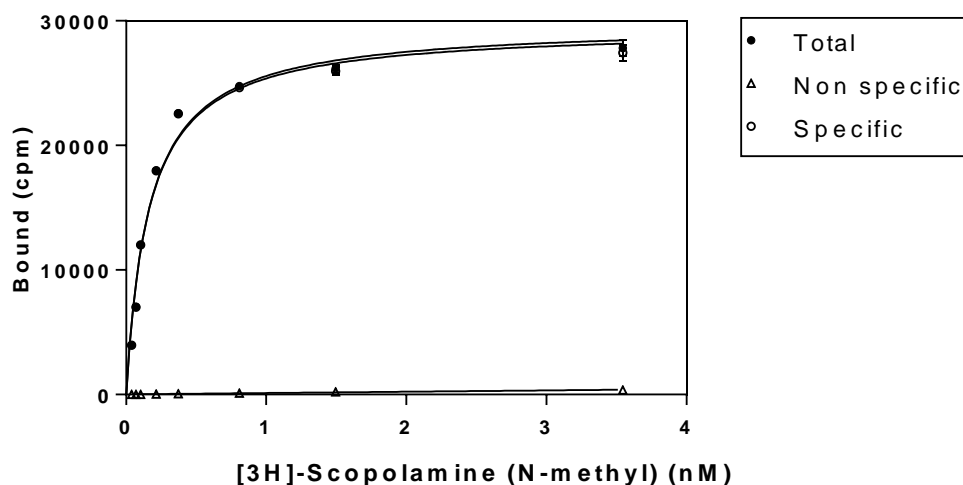


Figure 1: Saturation binding assay curve (filtration)

96-well saturation binding assay curve (35 μ g membranes/well, TopCount $^{\circ}$) using [3 H]-Scopolamine (N-methyl) (PerkinElmer NET636 Lot No.: 1635216).

Typical Product Data

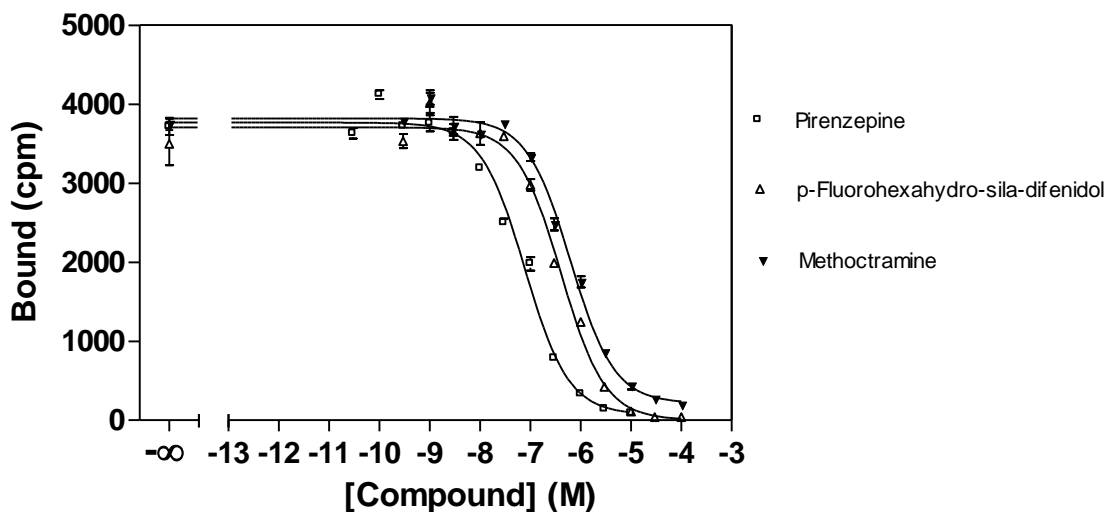


Figure 2: Competition binding assay curve (filtration)

96-well competition binding assay curve (35 μ 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 _i (nM)
Pirenzepine	26
p-Fluorohexahydro-sila-difenidol	138
Methoctramine	213

Suggested Materials and Instrumentation

Please visit our website

www.perkinelmer.com/GPCR

This product is not for resale or distribution except by authorized distributors.

LIMITED WARRANTY: PerkinElmer BioSignal Inc. warrants that, at the time of shipment, the products sold by it are free from defects in material and workmanship and conform to specifications which accompany the product. PerkinElmer BioSignal Inc. makes no other warranty, express or implied with respect to the products, including any warranty of merchantability or fitness for any particular purpose. Notification of any breach of warranty must be made within 60 days of receipt unless otherwise provided in writing by PerkinElmer BioSignal Inc. No claim shall be honored if the customer fails to notify PerkinElmer BioSignal Inc. within the period specified. The sole and exclusive remedy of the customer for any liability of PerkinElmer BioSignal Inc. of any kind including liability based upon warranty (express or implied whether contained herein or elsewhere), strict liability contract or otherwise is limited to the replacement of the goods or the refunds of the invoice price of goods. PerkinElmer BioSignal Inc. shall not in any case be liable for special, incidental or consequential damages of any kind.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

Copyright ©2009, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.