PerkinElmer Life Sciences, Inc.
549 Albany Street
Boston, MA 02118

Technical Data
Certificate of Analysis

PRODUCT INFORMATION SHEET

RBHM1M : Human M1 Muscarinic Receptor

SPECIFIC LOT DATA: Lot 1996

Receptor Concentration (B_{max}): 1.6 pmol/mg protein

K_d for [^{3}H]NMS Binding: 0.78 nM

Volume: 1 ml per vial; 400 microassays/vial
0.25 ml per vial; 100 microassays/vial

Protein Concentration: 9.7 mg/ml

PACKAGING: Membranes suspended in 50 mM TRIS-HCl pH 7.4, 10% sucrose.

RECOMMENDED ASSAY PROCEDURES:

Thaw: Thaw vials rapidly; dilute with binding buffer; homogenize. Keep on ice.
Incubation buffer: Phosphate buffered saline (PBS), pH 7.4

Binding protocol for 400 microassays per vial: Incubate 24.25 µg of membranes (20 µl of a 1:8 dilution), buffer and [^{3}H]NMS (0.38 nM, 81 Ci/mmol), in a total volume of 0.2 ml for 120 min at 25°C. Non-specific binding was determined with 5 µM atropine. Filter over GF/C filter (presoaked in 0.5% PEI), wash filter 9x with 0.5 ml ice-cold 50 mM Tris-HCl, 0.9% NaCl, pH 7.4. Under these conditions, we obtained 1541 cpm of total binding, of which, 10 cpm were non-specific. **NOTE :** ligand depletion occurs under these conditions, significantly altering Bmax and K_d calculations. The Bmax and K_d values were calculated using the conditions named above with only 8 µg of membranes. A final QC point using 24.25 µg of membranes was used to determine the counts quoted.

STABILITY AND STORAGE RECOMMENDATIONS: Storage at -80°C is recommended. Long term stability of this product is under investigation. Repeated freeze-thawing of this product is not recommended.

ORIGIN: Human m1 muscarinic receptors transfected in CHO-K1 cells [NJ Buckley, TI Bonner, CM Buckley, MR Brann *Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells*. Mol. Pharm. **35**: 469-476, 1989]

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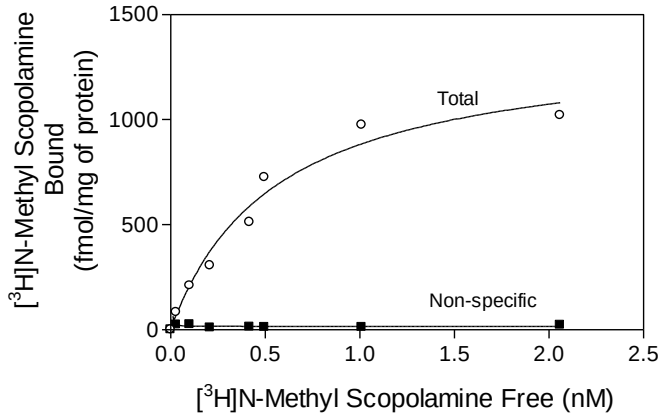
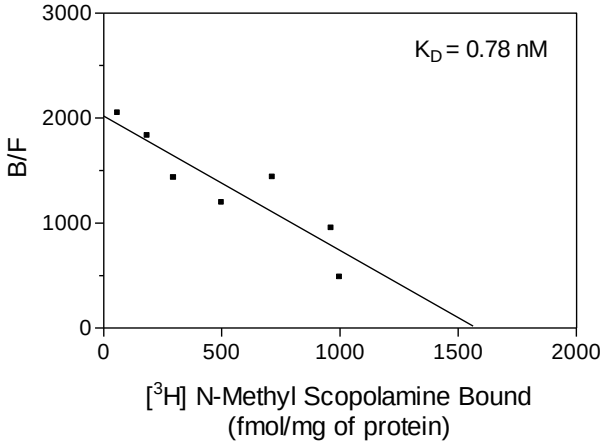
ReceptorBiology™

Worldwide Headquarters: PerkinElmer Life Sciences, Inc., Boston, MA. 02118-2512 U.S.A. (800) 551-2121
European Headquarters: PerkinElmer Life Sciences, Inc., B-1930 Zaventem, Belgium +32 2 717 7924
http://www.perkinelmer.com

[^{3}H] NMS BINDING TO HUMAN MUSCARINIC M1 RECEPTOR
Lot : 1996





Affinity constants at M1 muscarinic receptor, Lot 1996

Competitor	K_i (nM)
p-F-HHSID	146
Pirenzepine	37
Methoctramine	340