

Technical Data Certificate of Analysis

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

Membrane Target Systems™ human Muscarinic M₁ Receptor

PRODUCT No.: RBHM1M400UA

LOT No.: 452-860-A

MATERIAL PROVIDED

MEMBRANES: 1 x 400 units / 1000 µl frozen aliquot.
PACKAGING BUFFER: 50 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 10 mM MgCl₂, 10% sucrose.

PRODUCT INFORMATION

CELLULAR BACKGROUND: CHO-K1
PROTEIN ACCESSION NUMBER: NM_000738
UNIT SIZE: 35 µg protein / unit
STORAGE CONDITIONS: Store at -80°C. Avoid repeated freeze-thaw.
STABILITY: This product is stable for at least 3 years from reception if used and stored under recommended conditions.

QUALITY CONTROL

EXPRESSION LEVEL (B_{Max}): 1.39 pmol/mg membrane protein.
K_D FOR [³H]-SCOPOLAMINE (N-METHYL) : 0.36 nM
PROTEIN CONCENTRATION: 14 mg/ml

RECOMMENDED ASSAY CONDITIONS

ASSAY BUFFER: PBS pH 7.4
WASH BUFFER: 50 mM Tris-HCl pH 7.4, 0.9 % NaCl
BINDING PROTOCOL: Binding assays are performed in 550 µl 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 µl of incubation buffer or unlabeled ligand (Atropine, 5 µM final for non specific binding)
25 µl of radioligand at the appropriate concentration (see graph on page 2)
500 µl of diluted membranes
- 3 - Incubation time: 120 minutes at 27 °C
- 4 - Filtration: Over GF/C filter presoaked in 0.5 % PEI then washed 9x with 500 µl of ice cold wash buffer.

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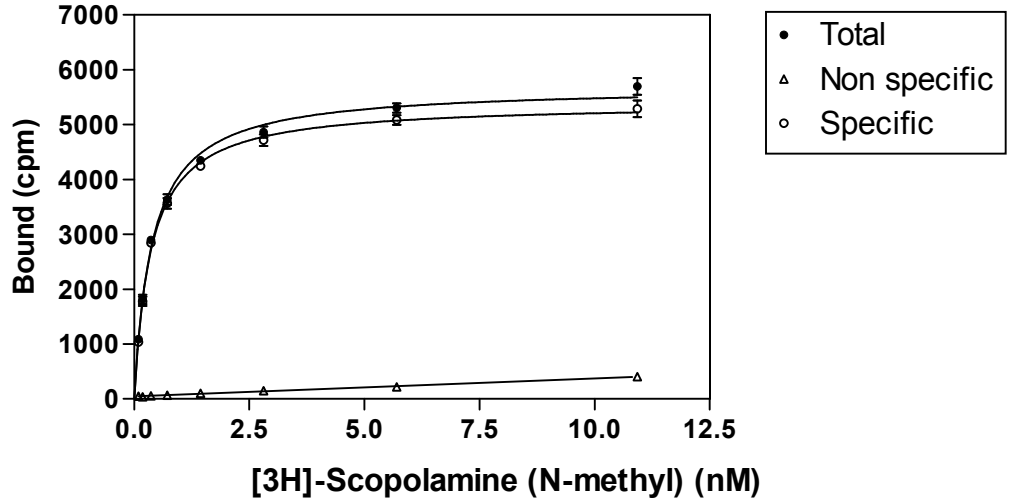


Figure 1: SATURATION BINDING ASSAY CURVE (FILTRATION)
96-well saturation binding assay curve (35 µg membranes/well, TopCount™).

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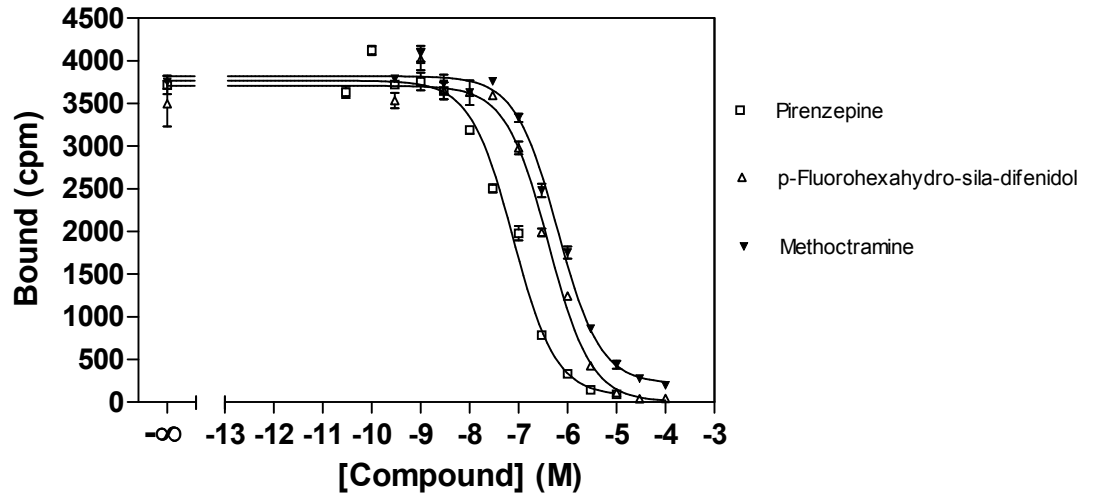


Figure 2: COMPETITION BINDING ASSAY CURVE (FILTRATION)
96-well competition binding assay curve (35 µg membranes/well, TopCount™). Recommended radioligand concentration = 0.2 nM.

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

Reference Compounds	Ki (nM)
Pirenzepine	26
p-Fluorohexahydro-sila-difenidol	138
Methoctramine	213

SUGGESTED MATERIALS AND INSTRUMENTATION

	SUPPLIER	CATALOG #
PHARMACOLOGY ASSAYS		
• [³ H]-Scopolamine (N-methyl)	PerkinElmer	NET636
• Atropine	Sigma	A025
• Unifilter-96 GF/C	PerkinElmer	6005174
• Unifilter-96 GF/B	PerkinElmer	6005177
• Filtermat A GF/C	PerkinElmer	1450-421
INSTRUMENTS		
• TopCount™	PerkinElmer	visit the web site or contact your local sales office
• Microbeta®	PerkinElmer	visit the web site or contact your local sales office
• FilterMate Unifiter 96-Harvester	PerkinElmer	C961962

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