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PRODUCT DATASHEET

ChemiScreen™ M_4 Muscarinic Acetylcholine Membrane Preparation

CATALOG NUMBER:	HTS117M	**QUANTITY:**	200 units
LOT NUMBER:	SC20181211	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: The muscarinic acetylcholine receptor (mAChR) family consists of five GPCRs that mediate some of the neurotransmission functions of acetylcholine in the CNS and the periphery. The M_1, M_3 and M_5 receptors couple to Gq to mobilize intracellular calcium, whereas the M_2 and M_4 receptors couple to Gi/o to inhibit cAMP production (Caulfield and Birdsall, 1998). Muscarinic M_4 receptor is known to be abundantly expressed in the striatum (Levey 1993). Consistent with this fact, locomotor activity was significantly increased in M_4 receptor-deficient mice (Gomeza et al. 1999). M_4 membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of agonists and antagonists of M_4.

APPLICATIONS: Radioligand binding assay

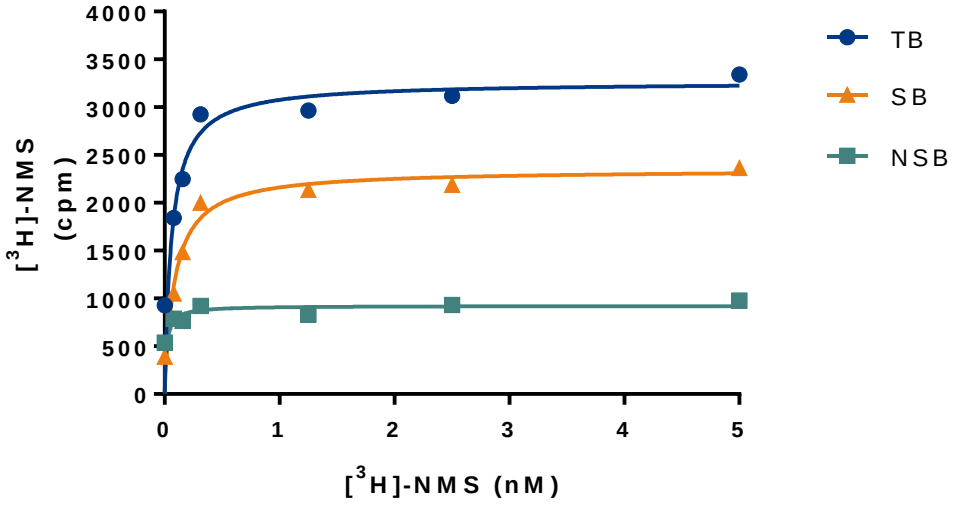

Figure 1. Saturation binding for M_4. 10 µg/well M_4 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled NMS in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled PD 102807. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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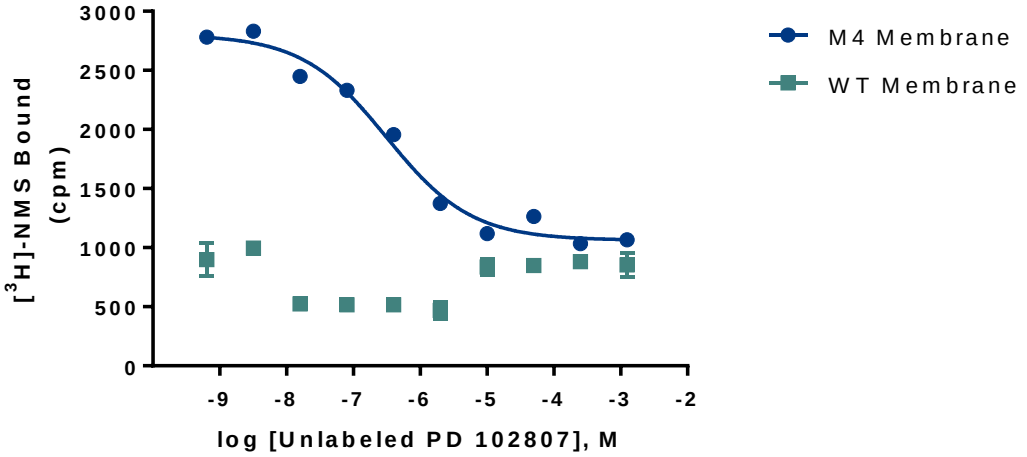

Figure 2. Competition binding for M_4. 10 µg/well M_4 Membrane Preparation and 10 µg/well wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.4 nM ^{3}H-labeled NMS and increasing concentrations of unlabeled PD 102807. More than 2.5-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{3}H]-NMS binding: 2.64 pmol/mg protein
K_d for [^{3}H]- NMS binding: ~0.09 nM
Signal:background: >2.5-fold

TRANSFECTION: Human M4 cDNA (Accession Number: NM_00741)

Species: Human

HOST CELLS: Chem-4, an adherent cell line expressing the promiscuous G-protein, Gα15.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C.

Radioligand: [^{3}H]-NMS. (Perkin Elmer#:NET636)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 2.5-fold signal:background with [^{3}H]-NMS.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

1. Caulfield MP and Birdsall NJM (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev. 50: 279-290.
2. Levey AI (1993). Immunological localization of M1-M5 muscarinic acetylcholine receptors in peripheral tissue and brain. Life Sci. 52: 441-448.
3. Gomeza J et al. (1999) Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M4 muscarinic acetylcholine receptor knockout mice. Proc. Natl. Acad. Sci. USA 96: 10483-10488.