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PRODUCT DATASHEET

ChemiScreen™ H_1 HISTAMINE Membrane Preparation

CATALOG NUMBER:	HTS050M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND:

Histamine exerts its biological effects through a family of four GPCRs. Stimulation of the H_1 histamine receptor causes smooth muscle contraction, induces vascular permeability, and stimulates catecholamine release from the adrenal medulla. H_1 is also widely expressed in the brain, and activation of H_1 excites several types of neurons by blocking potassium conductance (Hill *et al.,* 1997). H_1 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 antagonists of H_1 interactions and its ligands. The membrane preparations exhibit a Kd of 4.5 nM for [^{3}H]-Pyrilamine. With 3 nM [^{3}H]-Pyrilamine, 10 µg/well of H_1 Membrane Prep typically yields a greater than 4-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

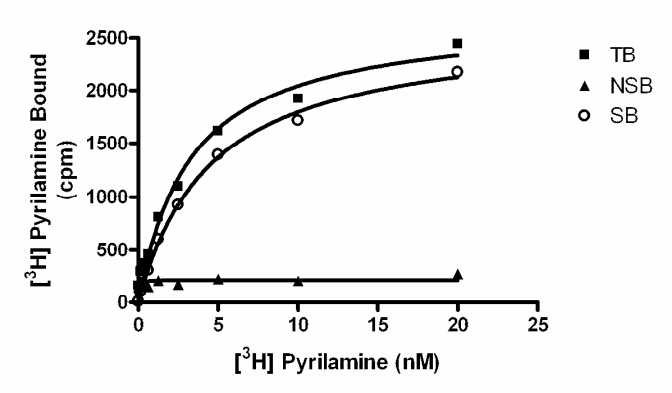

Figure 1. Saturation Binding for H_1. 10 µg/well of H_1 Membrane Preparation were incubated with increasing amounts of [^{3}H]-Pyrilamine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Pyrilamine. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC.

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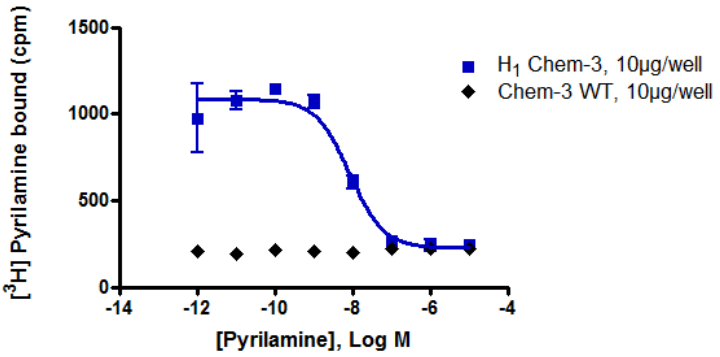

Figure 2. Competition Binding for H_1. 10 µg/well of H_1 or Wild Type Chem-3 (Catalog # HTS000MC3) Membrane Preparation were incubated with 3 nM [^{3}H]-Pyrilamine and increasing concentrations of unlabeled Pyrilamine and were subjected to filtration binding. More than a 4-fold signal:background ratio was obtained. The data are from a representative sample of lot SC.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{3}H]-Pyrilamine Binding: 8.3 pmol/mg protein
K_d for [^{3}H]-Pyrilamine Binding: 4.5 nM
Signal:Background: ≥4-fold

TRANSFECTION: Full-length human HRH1 cDNA encoding H_1 (Accession Number: NM_000861)

HOST CELLS: Chem-3, a suspension mammalian cell line without any endogenous H_1 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate, and incubated for 2 h at room temperature. Prior to filtration, an FC 96-well harvest plate was coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions were transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted for determination of receptor-associated radioligand binding.

Binding Buffer: 50 mM Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, filtered and stored at 4°C.

Radioligand: [^{3}H]-Pyrilamine (PerkinElmer#: NET594)

Wash Buffer: 50 mM Tris, pH 7.4, 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 a 4-fold signal:background ratio with [^{3}H]-Pyrilamine.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol, and 1% BSA no preservatives.

Membranes proteins were adjusted to 2 mg/mL in packaging buffer, dispensed at 1 mL per vial, 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Hill SJ *et al.* (1997). International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.* 49:253-278.