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PRODUCT DATASHEET

ChemiScreen™ Recombinant Human H_2 Histamine Receptor Membrane Preparation

CATALOG NUMBER:	HTS086M	**QUANTITY:**	200 units
LOT NUMBER:	22G2709	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: The histamine H_2 receptor is a widely distributed G-protein coupled receptor, which is important in regulating a host of physiologic actions extending from gastric acid secretion to gastrointestinal motility (Del Valle and Gantz, 1997). Histamine mediated activation of its receptor leads to signaling through both adenylate cyclase and phosphoinositide/protein kinase C second messenger systems (Hill, 1990). Antagonists for this receptor have proven to be effective therapy for acid peptic disorders of the GI tract. Certain antagonists are used in the treatment of neuropsychiatric and neurological diseases e.g. schizophrenia, Alzheimer's disease, and Parkinson's disease. EMD Millipore's H_2 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 at H_2.

APPLICATIONS: Radioligand binding assay

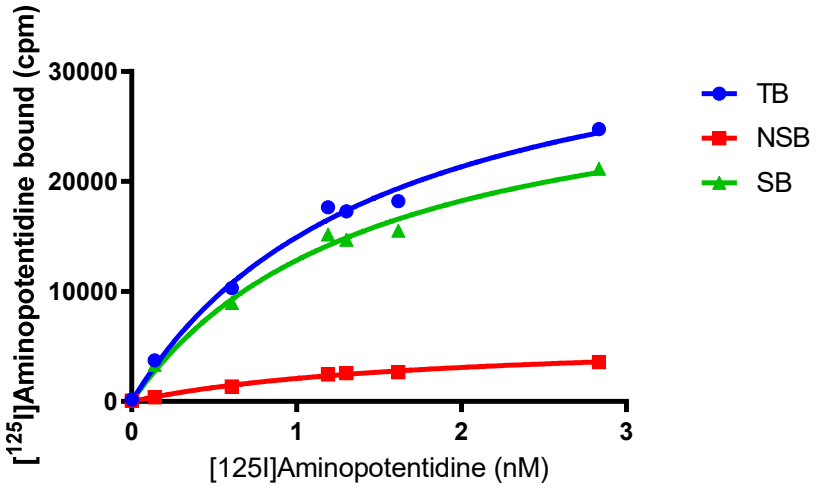

Figure 1. Saturation binding for H_2. 5 µg/well H_2 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled aminopotentidine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 1000-fold excess unlabeled tiotidine. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

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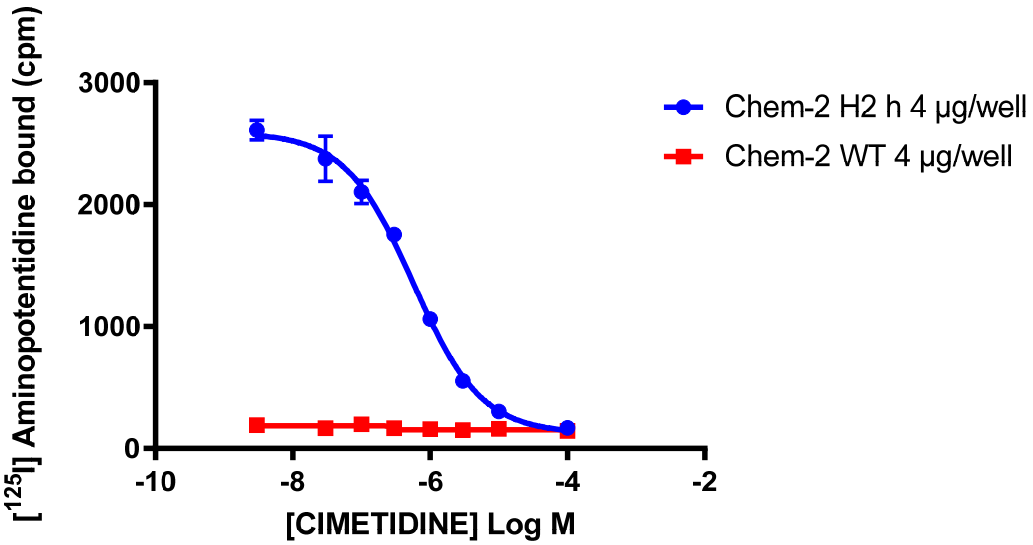

Figure 2. Competition binding for H_2. 4 µg/well H_2 Membrane Preparation and wild-type Chem-2 Membrane Preparation (EMD Millipore catalog # HTS000MC2) were incubated in a 96-well plate with 0.65 nM ^{125}I-labeled Iodoaminopotentidine and increasing concentrations of unlabeled cimetidine. More than 7- fold signal:background was obtained with cimetidine. Sample data from a representative lot.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{125}I] Iodoaminopotentidine binding: 2.3 pmol/mg protein
K_d for [^{125}I] Iodoaminopotentidine binding: ~1.4 nM
Signal:background: ≥7-fold

TRANSFECTION: Full-length human HRH2 cDNA encoding H_2 (Accession Number: NM_022304)

Species: Human

HOST CELLS: Chem-2, a suspension mammalian cell line with minimum amount of endogenous H_2 expression.

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 50mM 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: [[^{125}I] Iodoaminopotentidine (Amersham#**:** IM264)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM 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 7-fold signal:background with ^{125}I-labeled Iodoaminopotentidine at 0.65 nM.

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. Del Valle, J., and Gantz, I. (1997) Novel insights into histamine H2 receptor biology. Am. J. Physiol. 273, G987–G996.
2. Hill, S. J. (1990) Distribution, properties, and functional characteristics of three classes of histamine receptor. Pharmacol. Rev. 42, 45–83.