

PRODUCT DATASHEET

ChemiScreen™ D₁ Dopamine Membrane Preparation

CATALOG NUMBER: HTS102M **QUANTITY:** 200 units
LOT NUMBER: 21E1210 **VOLUME/CONCENTRATION:** 1 mL, 2.0 mg/mL

BACKGROUND: Dopamine exerts its action by binding to five distinct dopamine receptors, all of which belong to G protein-coupled receptor family (Missale et al. 1998). The D₁ subtype is the most abundant dopamine receptor in the central nervous system. Activation of D₁ receptor stimulates adenylyl cyclase, activates cyclic AMP-dependent protein kinases. It regulates neuronal growth and development, mediate some behavioral responses and modulate dopamine receptor D₂-mediated events. D₁ 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 D₁ interactions and its ligands.

APPLICATIONS: Radioligand Binding Assay

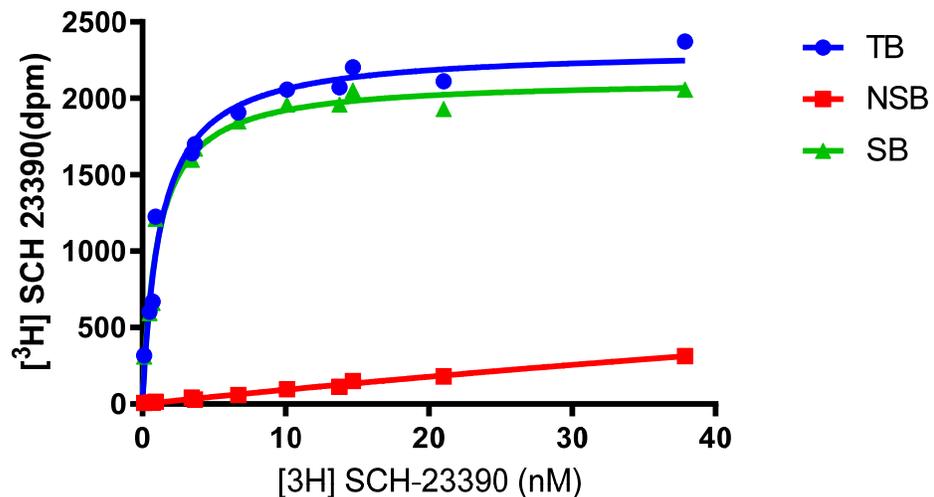


Figure 1. Saturation Binding for D₁. 10 µg/well of D₁ Membrane Preparation was incubated with increasing amount of [³H] SCH-23390 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled SCH-23390. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

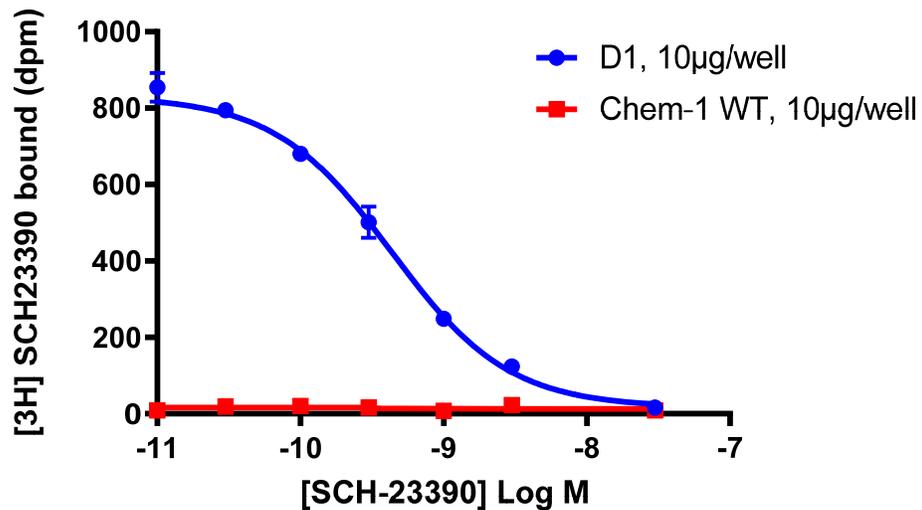


Figure 2. Competition Binding for D₁. 10 µg/well of D₁ Membrane Preparation (HTS102M) was incubated with 1.5 nM [³H] SCH-23390 and increasing concentrations of unlabeled SCH-23390. More than 10-fold signal:background ratio was obtained. Sample data from a representative lot.

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [³H] SCH-23390 binding: 27.3 pmol/mg protein
 K_d for [³H] SCH-23390 binding: 1.0 nM

Species: Full-length human DRD1 cDNA (Accession Number: NM_000794).

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous D₁ 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, a GF/C 96-well filter plate 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 Tris-HCl (pH 7.4), 5 mM KCl, 5 mM MgCl₂, 1.5 mM CaCl₂ and 5 mM EDTA, filtered and stored at 4°C.

Radioligand: [³H] SCH-23390 (PerkinElmer#:NET-930)

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 10-fold signal:background with [³H] SCH 23390 at 1.5 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Missale C *et al.* (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78:189-225.

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