

PRODUCT DATASHEET
ChemiScreen™ D₅ Dopamine Membrane Preparation

CATALOG NUMBER:	HTS129M	QUANTITY:	200 units
LOT NUMBER:	SC872491	VOLUME/CONCENTRATION:	1 mL, 2.0 mg/mL

BACKGROUND: Dopamine is a catecholamine neurotransmitter that functions in the CNS to control locomotor, cognitive, emotional and neuroendocrine processes, and in the periphery to modulate cardiovascular, renal and gastrointestinal processes. The biological activities of dopamine are mediated by a family of five GPCRs. The D₁ and D₅ subtypes couple to G_s to increase intracellular cAMP, whereas the D₂, D₃ and D₄ subtypes couple to G_i to reduce cAMP (Missale *et al.*, 1998). The hypertensive phenotype of mice with a targeted deletion of D₅ indicates that D₅ regulates central control of sympathetic vascular tone (Hollon *et al.*, 2002). In addition, D₅ modulates locomotion and corticostriatal long-term depression (Centonze *et al.*, 2003). The cloned human D₅-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant D₅ expression on the cell surface and contains high levels of the promiscuous G protein G α 15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between D₅ and its ligands. The membrane preparations exhibit a K_d of 4.5 nM for [³H]-SCH 23390. With 0.5 nM [³H]-SCH 23390, 10 μ g/well D₅ Membrane Prep yields greater than 3-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

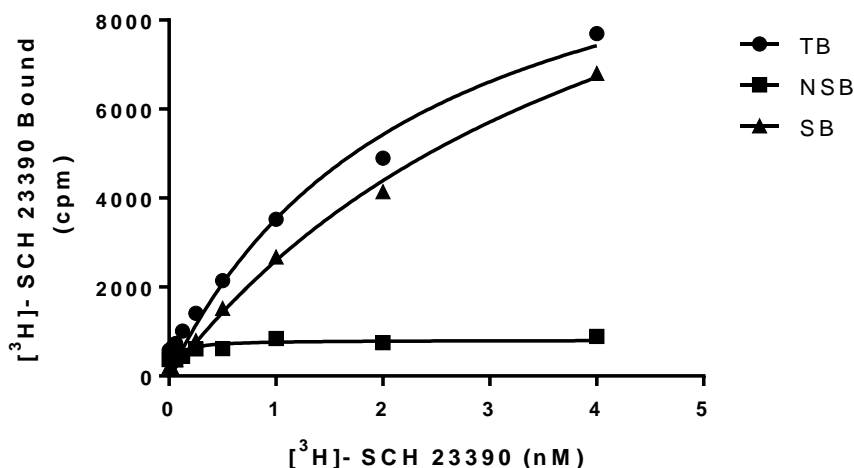


Figure 1. Saturation binding for D₅. 10 μ g/well D₅ Membrane Preparation was incubated with increasing amount of ³H labeled SCH 23390 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled SCH 23390. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot SC872491

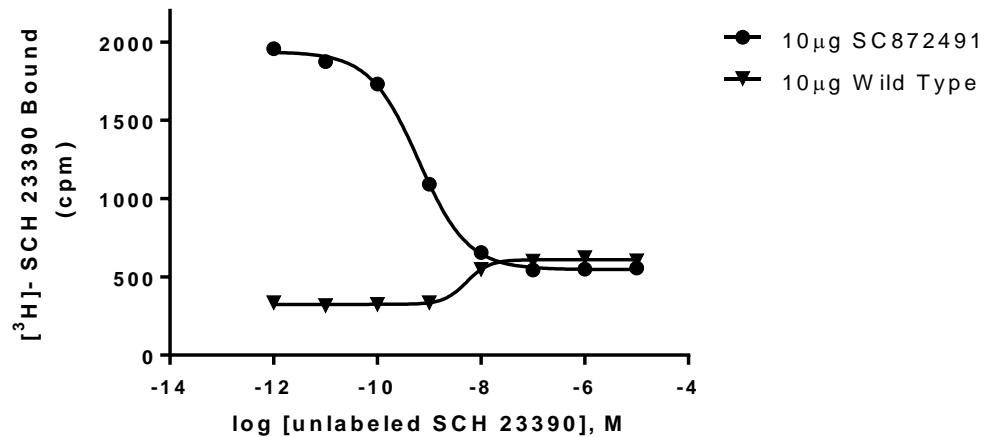


Figure 2. Competition binding for D₅. D₅ Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1), each at 10 µg/well in a 96-well plate, were incubated with 0.5 nM ³H labeled SCH 23390 and increasing concentrations of unlabeled SCH 23390, and subjected to filtration binding. Sample data from a representative lot SC872491

SPECIFICATIONS: 1 unit = 10 µg

B_{max} for [³H] SCH 23390 binding: 16.0pmol/mg protein

K_d for [³H] SCH 23390 binding: 4.5 nM

TRANSFECTION: Full-length human DRD5 cDNA encoding D₅ (Accession Number: NM_000798)

HOST CELLS: Chem-1, an adherent mammalian cell line with no endogenous D₅ 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 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C.

Radioligand: [³H]-SCH 23390. (Perkin Elmer#:NET-930)

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 3-fold signal:background with ³H labeled SCH 23390 at 0.5 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. Centonze D *et al.* (2003) Distinct roles of D₁ and D₅ dopamine receptors in motor activity and striatal synaptic plasticity. *J. Neurosci.* 23: 8506-8512.
2. Hollon TR *et al.* (2002) Mice lacking D₅ dopamine receptors have increased sympathetic tone and are hypertensive. *J. Neurosci.* 22: 10801-10810.

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