

PRODUCT DATASHEET

ChemiScreen™ D₃ Dopamine Membrane Preparation

CATALOG NUMBER: HTS103M **QUANTITY:** 200 units
LOT NUMBER: SC251143 **VOLUME/CONCENTRATION:** 1 mL, 1 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 dopamine D₃ receptor is expressed in phylogenetically older regions of the brain, suggesting that it plays a role in cognitive and emotional functions. It has been the target of potential drugs for psychotic disorders and Parkinson's disease. D₃ 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 D₃ interactions and its ligands.

APPLICATIONS: Radioligand Binding Assay

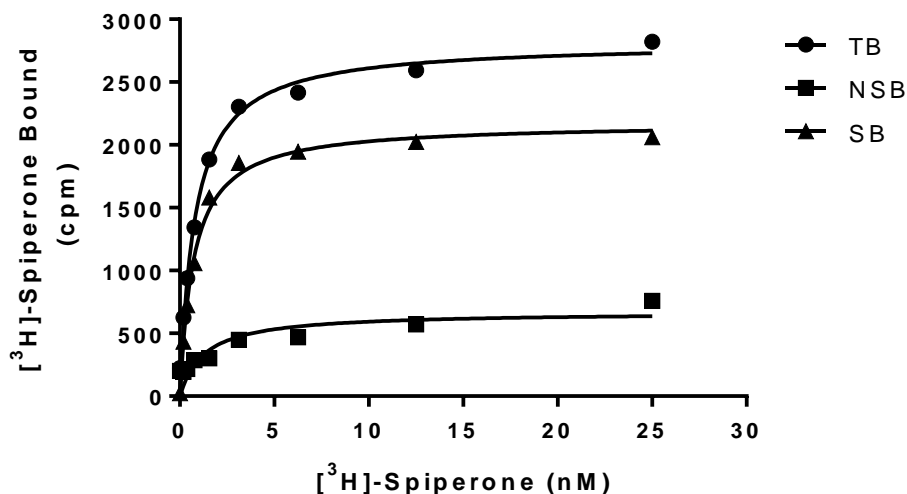


Figure 1. Saturation Binding for D₃ Receptor. 5 μg/well of D₃ Membrane Preparation was incubated with increasing amounts of [³H]-Spiperone in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Raclopride. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC251143.

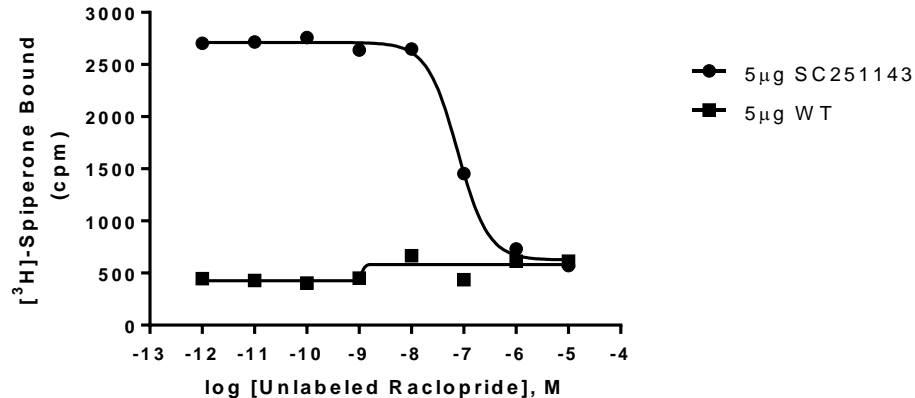


Figure 2. Competition Binding for D₃ Receptor. 5 µg/well of D₃ Receptor Membrane Preparation or Wild-Type Chem-1 Membrane Preparation (WT; Catalog # HTS000MC1) were incubated with 25 nM [³H]-Spiperone and increasing concentrations of unlabeled Raclopride, and more than a 4-fold signal:background ratio was obtained. The data are from a representative sample of lot SC251143.

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [³H]-Binding: 5.37 pmol/mg protein
 K_d for [³H]-Binding: 0.73 nM
 Signal:background: >4-fold

Species: Human D₃ Receptor (Accession number NM_000796).

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous D₃ Receptor 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. Prior to filtration, a GF/C 96-well filter plate was coated with 0.33% polyethylamine for 30 min, followed by being washed with 50 mM Tris, pH 7.4. 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.

Binding Buffer: 50 mM Tris, pH 7.4, 120 mM NaCl, filtered and stored at 4°C.

Radioligand: [³H]-Spiperone (PerkinElmer#: NET1187)

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 [³H]-Spiperone at 25 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 1 mg/mL 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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