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CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN D_2 DOPAMINE RECEPTOR

CATALOG NUMBER:	HTS039M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

Dopamine is a catecholamine neurotransmitter that functions in the CNS to control locomotor, cognitive, emotional and neurendocrine 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 D_1 and D_5 subtypes couple to G_s to increase intracellular cAMP, whereas the D_2, D_3 and D_4 subtypes couple to G_i to reduce cAMP (Missale *et al.*, 1998). The D_2 dopamine receptors have been of particular clinical interest due to their regulation of prolactin secretion and their affinity for antipsychotic drugs. The D_2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop (D_{2S} and D_{2L}) (Giros *et al.*, 1989; Grandy *et al.*, 1989). Millipore's D_{2L} dopamine receptor 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 for agonists and antagonists of the D_2 dopamine receptor. The membrane preparations exhibit EC50s of 16 nM for dopamine in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding Assay, Radioligand Binding Assay

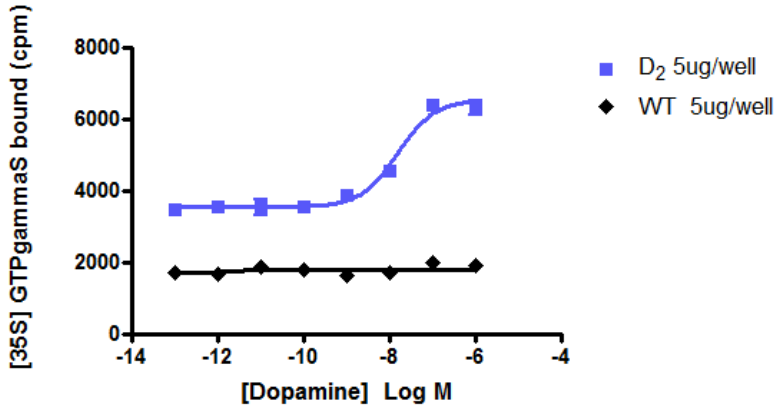

Figure 1. Binding of [^{35}S]-GTPγS to D_2 membrane preparation. 5 µg/well D_2 Membrane Preparation (catalog # HTS039M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled dopamine. Bound radioactivity was determined by filtration and scintillation counting.

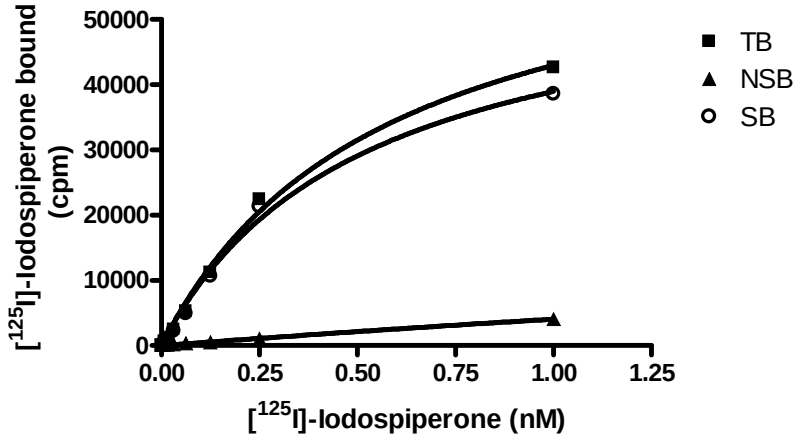

Figure 2. Saturation binding for D_2. 5 µg/well D_2 Dopamine Receptor Membrane Preparation was incubated with increasing amount of [^{125}I]-Iodospiperone in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled raclopride. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

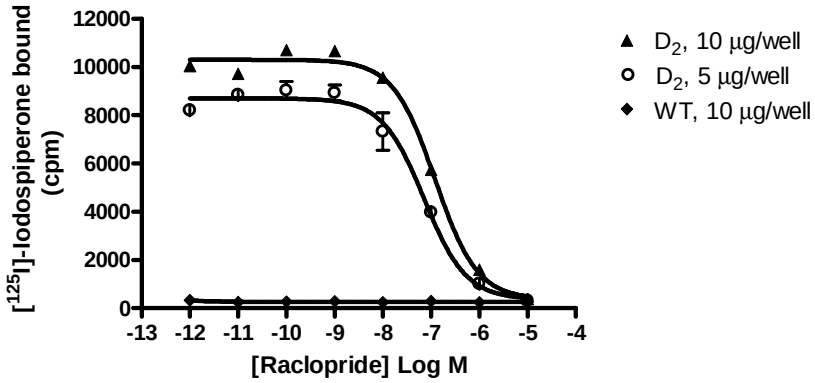

Figure 3. Competition binding for D_2 Dopamine Receptor. D_2 Dopamine Receptor Membrane Preparation (5 or 10µg/well) or Wild-Type Chem-1 membrane preparation (WT; Millipore Catalog # HTS000MC1) was incubated with 0.1 nM [^{125}I]-Iodospiperone and increasing concentrations of unlabeled Raclopride, and more than 10- fold signal:background was obtained. Sample data from a representative lot

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
EC50 in GTPγS binding assay by Dopamine: ~ 16 nM
Signal window: >2500 cpm

Human DRD2 encoding D_2 Dopamine Receptor long isoform (D_{2L}; Accession number NM_000795)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous D_2 Dopamine Receptor 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 (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM Tris, pH 7.4. 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, pH 7.4, 120 mM NaCl, filtered and stored at 4℃
Radioligand: [^{125}I] Iodospiperone (Perkin Elmer # NEX284)
Wash Buffer: 50 mM Tris, pH 7.4, filtered and stored at 4℃.

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 10-fold signal:background with ^{125}I-labeled Iodospiperone at 0.1 nM

PRESENTATION:

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

Packaging method: Membranes protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80^{o}C.

STORAGE/HANDLING:

Store at –70℃. Product is stable for at least 6 m onths from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

Grandy DK *et al.* (1989) Cloning of the cDNA and gene for a human D2 dopamine receptor. *Proc. Natl. Acad. Sci. U S A..* 86: 9762-6.

Giros B *et al.* (1989) Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature* 342: 923-6.

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

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION