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PRODUCT DATASHEET

ChemiScreen™ D_2 Dopamine Membrane Preparation

CATALOG NUMBER:	HTS039M	**QUANTITY:**	200 units
LOT NUMBER:	SC20180925	**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). 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.

APPLICATIONS:

$GTP\gamma S^{35}$ Functional Assay

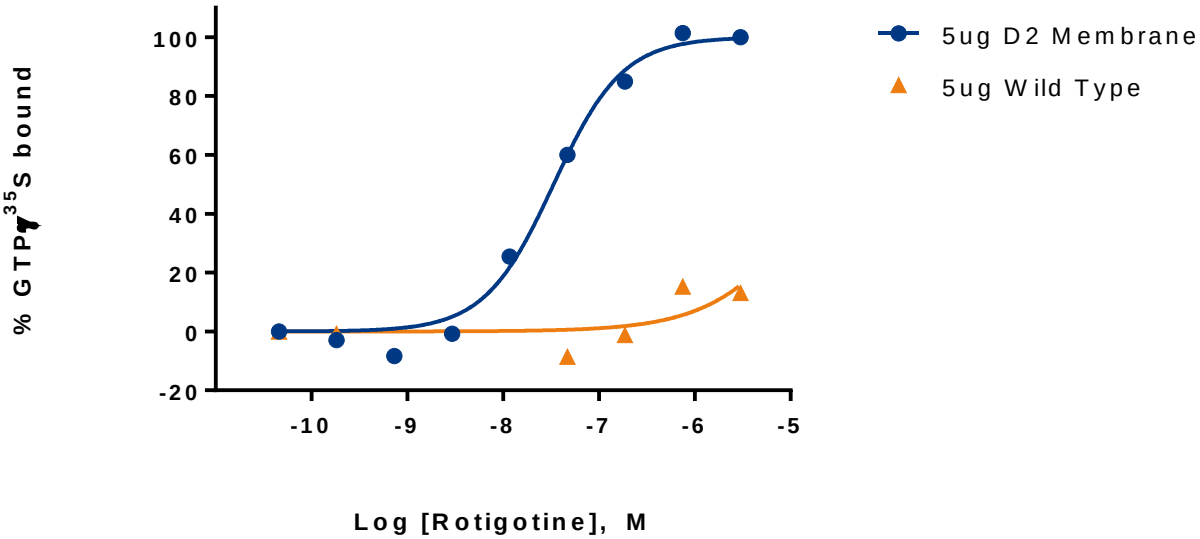

Figure 1. Binding of [^{35}S]-GTPγS to D_2 membrane preparation. 5 µg/well D_2 Membrane Preparation (catalog # HTS039M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled rotigotine. Bound radioactivity was determined by filtration and scintillation counting. Sample data from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

Eurofins Pharma Bioanalytics Services US Inc.

6 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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SPECIFICATIONS: 1 unit = 5 μg membrane preparation
EC50 in GTPγS binding assay by Rotigotine: ~33nM

SPECIES: 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 permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in assay buffer in a nonbinding 96-well plate. Unlabeled rotigotine added to the final concentration indicated in Figure 1 (final volume 100 µL), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (EMD Millipore MAHF B1H) previously prewetted with water, and washed 3 times (1 mL per well per wash) with cold wash buffer. The plate is dried and counted.

Binding buffer: 20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 0.5 µM GDP

Wash Buffer: 10 mM sodium phosphate, pH 7.4.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 1000 cpm specific dopamine-stimulated [^{35}S]-GTPγS binding.

Special Note: The D2 receptor membrane preparation is expected to be functional in a radioligand binding assay; however, the end user will need to determine the optimal radiolabeled ligand for use with this product.

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°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. 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.
2. Giros B *et al.* (1989) Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature* 342: 923-6
3. Missale C *et al.* (1998) Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189-225.
4. Wood M *et al.* (2014) Rotigtine is a potent agonist at dopamine D1 receptors as well as at dopamine D2 and D3 receptors. *BJP* 172: 1124-35.