

## human Dopamine D<sub>1</sub> Receptor

Product No.: ES-172-M400UA

Lot No.: 3424322

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### Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: S58541

Unit Size: 4 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl<sub>2</sub>, 10% sucrose.

Storage Conditions: Store at -80°C. Freeze-thaw is not recommended as it can affect product performance and homogeneity. In order to minimize negative impact of freeze-thawing, flash freeze in liquid nitrogen for 30 seconds prior to transferring to -80°C.

Stability: This product is stable for at least 3 years from reception if used and stored under recommended conditions.

### Quality Control

B<sub>max</sub> and K<sub>d</sub> are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method <sup>(1)</sup>. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B<sub>max</sub> in fmoles) by the maximal signal of a pre-defined reference tested in parallel. RTR is an indicator of lot-to-lot consistency. \*We certify that these results meet our quality release criteria.

Ratio-to-Reference (RTR): 0.6

Expression Level (B<sub>max</sub>): 2.7 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>3</sup>H]-SCH 23390: 0.06 nM

Protein Concentration: 4 µg/µL

(1) Smith, P.K., et al. (1985). *Anal. Biochem.* 150, 76-85.

## Recommended Assay Conditions

Assay Buffer:	50 mM Tris-HCl pH 7.4, 5 mM MgCl <sub>2</sub>
Wash Buffer:	50 mM Tris-HCl pH 7.4
Binding Protocol:	Binding assays are performed in 550 $\mu$ L total volume according to the following conditions:
1 - Membrane dilution:	0.05 mL of membranes + 24.95 mL assay buffer (1:500 dilution)
2 - Incubation:	25 $\mu$ L of incubation buffer or R(+)-SCH 23390 (Sigma D054) 20 $\mu$ M final for non-specific binding (Saturation binding assay) <i>For competition binding assay: 25 <math>\mu</math>L of reference compounds at decreasing concentrations (see figure 2)</i> 25 $\mu$ L of radioligand at the appropriate concentration (see graph below) 500 $\mu$ L of diluted membranes
3 - Incubation time:	60 minutes at 27°C
4 - Filtration:	aspirate and wash 9 x 500 $\mu$ L with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

## Lot Specific Data

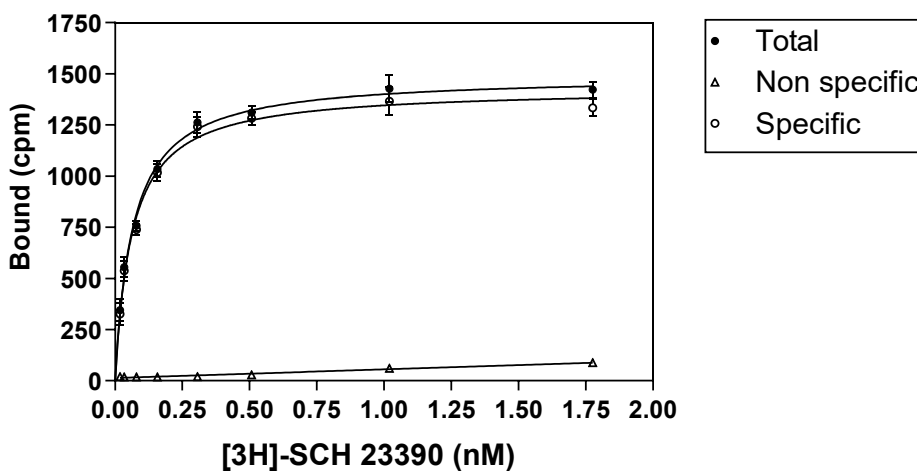


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (4  $\mu$ g membranes/well, TopCount®) using [<sup>3</sup>H]-SCH 23390 (Revvity NET930 Lot No.: 3369125)

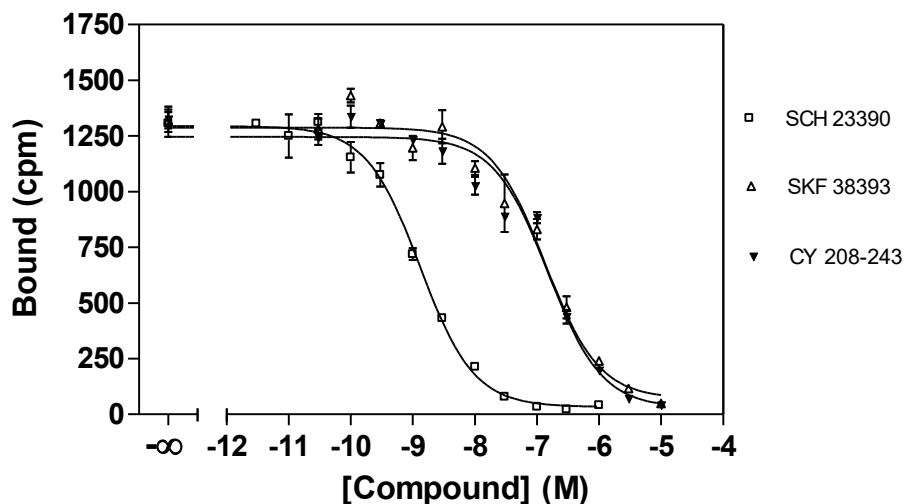


Figure 2: Competition binding assay curve (filtration)  
96-well competition binding assay curve (4 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.7 nM.

\*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

Reference Compounds	Ki (nM)
SCH 23390	0.36
SKF 38393	38
CY 208-243	42

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