human Dopamine Transporter

Product No.: RBHDATM400UA

Lot No.: 3350767

Material Provided

Membranes:	1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background:	CHO-K1
GenBank Accession Number:	NM_001044
Unit Size:	12 µg protein / unit
Storage Buffer:	50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM $MgCl_2$, 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_{max} and K_d are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method [1]. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B_{max} 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_{max}):	3.8 pmol/mg membrane protein.
K_d for [^{3}H]-WIN 35,428:	5.8 nM
Protein Concentration:	12 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, 100 mM NaCl
Wash Buffer:	50 mM Tris-HCl pH 7.4, 100 mM NaCl
Binding Protocol:	Binding assays are performed in 550 µL 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 µL of incubation buffer or GBR 12909 (Sigma D052) 10 µM final for non specific binding (Saturation binding assay) *For competition binding assay: 25 µL of reference compounds at decreasing concentrations (see figure 2)* 25 µL of radioligand at the appropriate concentration (see graph below) 500 µL of diluted membranes
3 - Incubation time:	120 minutes at 4°C
4 - Filtration:	aspirate and wash 9 x 500 µL with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

Lot Specific Data

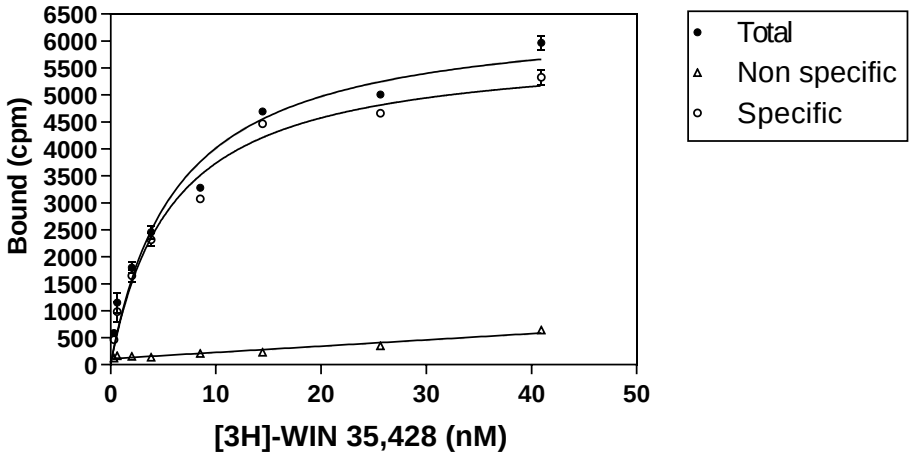

Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (12 µg membranes/well, TopCount®) using [^{3}H]-WIN 35,428 (Revvity NET1033 Lot No.: 3256736)

Revvity Proprietary Information

Typical Product Data

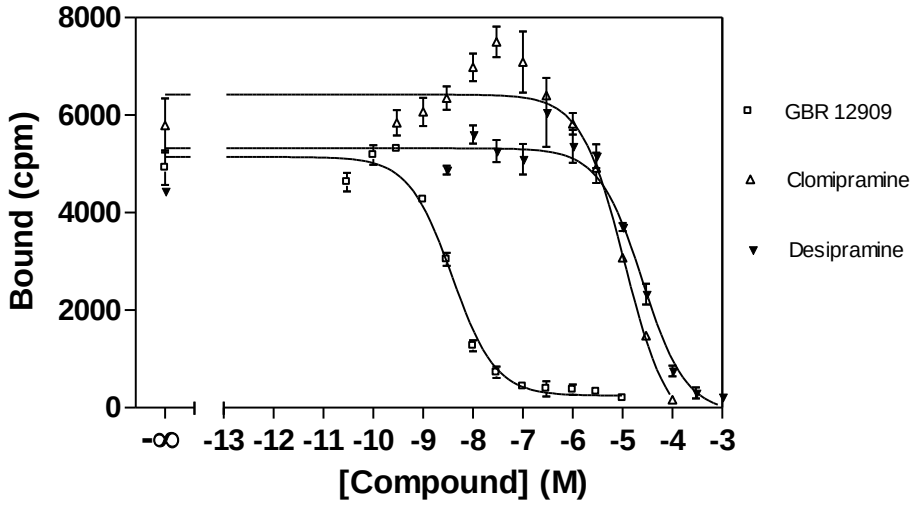

Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (12 µg membranes/well, TopCount®). Recommended radioligand concentration = 25 nM.

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

Reference Compounds	Ki (nM)
GBR 12909	1.5
Clomipramine	4369
Desipramine	9850

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