

human Dopamine Transporter

Product Number: RBHDATM400UA

Lot Number: 3241425

Material Provided

Membranes: 1 x 400 units / 400 μ L frozen aliquot

Product Information

Cellular Background: CHO-K1

GenBank Accession Number: NM_001044

Unit Size: 12 μ g protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl₂, 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): 1.2

Expression Level (B_{max}): 3.2 pmol/mg membrane protein.

K_d for [³H]-WIN 35,428 : 5 nM

Protein Concentration: 12 μ g/ μ L

(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 μ 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 μ L of incubation buffer or GBR 12909 (Sigma D052) 10 μ M final for non specific binding (Saturation binding assay)

For competition binding assay: 25 μ L of reference compounds at decreasing concentrations (see figure 2)

25 μ L of radioligand at the appropriate concentration (see graph below)
500 μ L of diluted membranes
 - 3 - Incubation time: 120 minutes at 4 $^{\circ}$ C
 - 4 - Filtration: aspirate and wash 9 x 500 μ L with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

Lot Specific Data

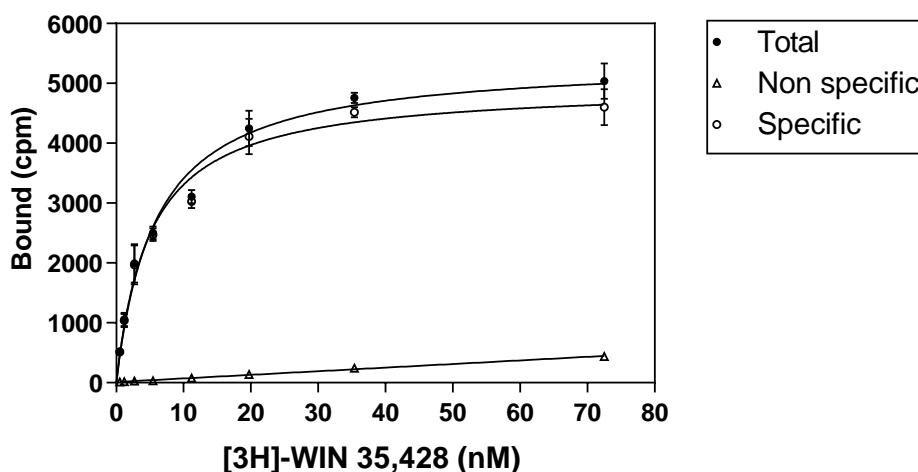


Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (12 μ g membranes/well, TopCount[®]) using [³H]-WIN 35,428 (Revvity NET1033 Lot No.: 3148009)

Typical Product Data

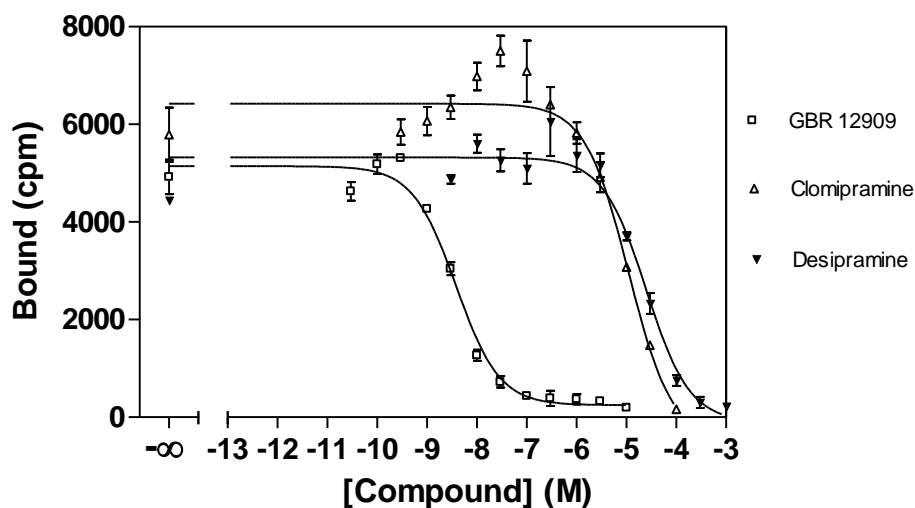


Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (12 μ g membranes/well, TopCount®). Recommended radioligand concentration = 25 nM.

*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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