

## human Serotonin Transporter

Product No.: RBHSTM400UA

Lot No.: 3103298

### Material Provided

Membranes: 1 x 400 units / 400  $\mu$ L frozen aliquot

### Product Information

Cellular Background: HEK293

GenBank Accession Number: NM\_001045

Unit Size: 9  $\mu$ g 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): 1.4

Expression Level (B<sub>max</sub>): 8.5 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>3</sup>H]-Imipramine : 0.7 nM

Protein Concentration: 9  $\mu$ g/ $\mu$ 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, 120 mM NaCl, 5 mM KCl
Wash Buffer:	50 mM Tris-HCl pH 7.4, 154 mM NaCl
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 Imipramine (Sigma I0899) 200 $\mu$ 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:	30 minutes at 27 $^{\circ}$ 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

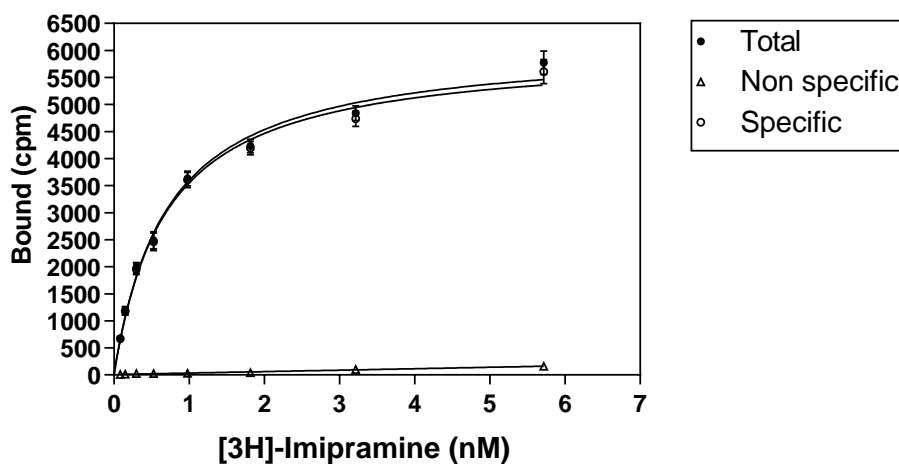


Figure 1: Saturation binding assay curve (filtration)  
 96-well saturation binding assay curve (9  $\mu$ g membranes/well, TopCount<sup>®</sup>) using [<sup>3</sup>H]-Imipramine (Revvity NET576 Lot No.: 3104081)

Typical Product Data

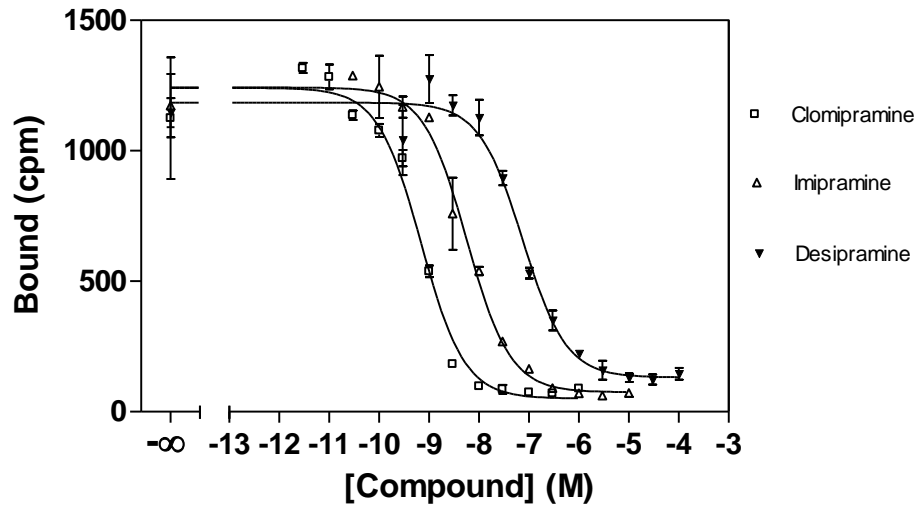


Figure 2: Competition binding assay curve (filtration)  
 96-well competition binding assay curve (9  $\mu$ g membranes/well, TopCount®). Recommended radioligand concentration = 2 nM.

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

Reference Compounds	Ki (nM)
Clomipramine	0.53
Imipramine	4.4
Desipramine	57

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