

Research use only. Not for use in diagnostic procedures.

Membrane Target Systems™

human Serotonin 5-HT_{1A} Receptor

Product No.: RBHS1AM400UA

Lot No.: 2838165

Material Provided

Membranes: $1 \times 400 \text{ units} / 1000 \mu \text{L}$ frozen aliquot

Product Information

Cellular Background: HEK293-EBNA

GenBank Accession Number: NM_000524 (K418N)

Unit Size: 16 μg 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): 1.47

Expression Level (B_{max}): 2.2 pmol/mg membrane protein.

 K_d for [3 H]-8-Hydroxy-DPAT : 0.63 nM

Protein Concentration: 6.4 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 MgSO₄

Wash Buffer: 50 mM Tris-HCl pH 7.4

Binding Protocol: Binding assays are performed in 550 µL total volume according to the

following conditions:

1 - Membrane dilution: 0.125 mL of membranes + 24.875 mL assay buffer (1:200 dilution)

2 - Incubation: 25 μL of incubation buffer or Serotonin (5-HT) (Sigma H9523) 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)

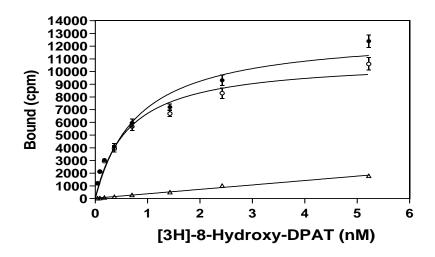
500 µL of diluted membranes

3 - Incubation time: 120 minutes at 37 °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



Non specificSpecific

Total

Figure 1: Saturation binding assay curve (filtration) 96-well saturation binding assay curve (16 µg membranes/well, TopCount®) using [³H]-8-Hydroxy-DPAT (Revvity NET929 Lot No.: 2789816)



Typical Product Data

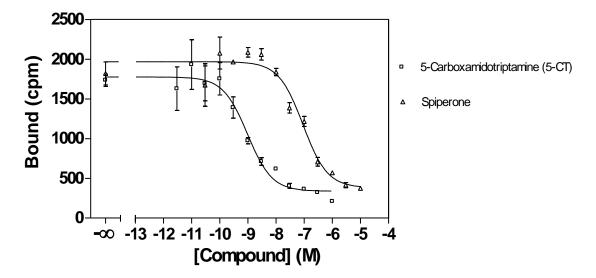


Figure 2: Competition binding assay curve (filtration) 96-well competition binding assay curve (16 μ g membranes/well, TopCount®). Recommended radioligand concentration = 2 nM.

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

Reference Compounds	Ki
	(nM)
5-Carboxamidotriptamine (5-CT)	0.57
Spiperone	53

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