

human Serotonin 5-HT₇ Receptor

Product No.: 6110512400UA

Lot No.: 2436929

Material Provided

Membranes: 1 x 400 units / 1000 µL frozen aliquot

Product Information

Cellular Background: CHO-K1

GenBank Accession Number: NM_000872 (Δ442GH, D444R)

Unit Size: 17 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): 0.7

Expression Level (B_{max}): 1.4 pmol/mg membrane protein.

K_d for [³H]-LSD: 0.92 nM

Protein Concentration: 6.8 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, 10 mM MgSO ₄ , 0.5mM EDTA
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 Clozapine (Sigma C6305) 25 µM final for non-specific binding (Saturation binding assay)
	<i>For competition binding assay: 25 µL of reference compounds at decreasing concentrations (see figure 2)</i>
	25 µL of radioligand at the appropriate concentration (see graph below) 500 µL of diluted membranes
3 - Incubation time:	120 minutes at 27 °C
4 - Filtration:	aspirate and wash 9 x 500 µL with ice cold wash buffer over GF/C filter (presoaked in 0.3% PEI).

Lot Specific Data

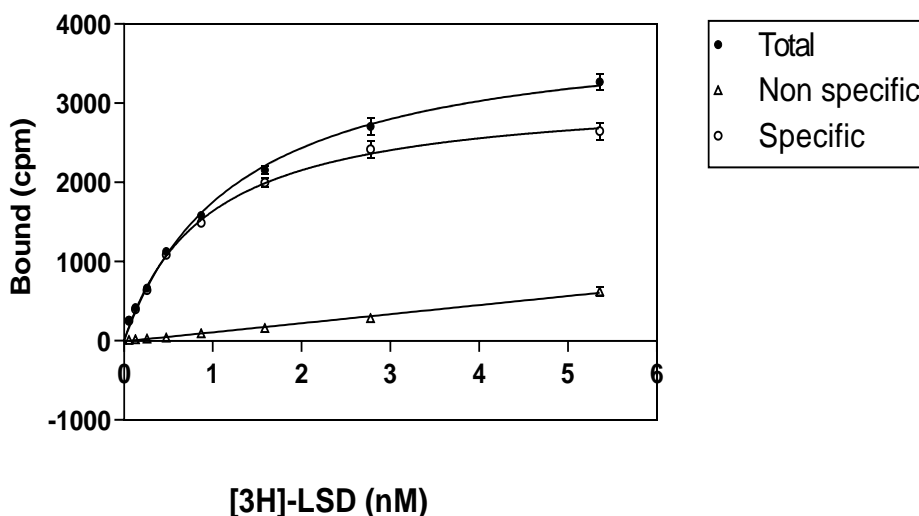


Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (17 µg membranes/well, TopCount®) using [³H]-LSD (Revvity NET638 Lot No.: 2435251)

Typical Product Data

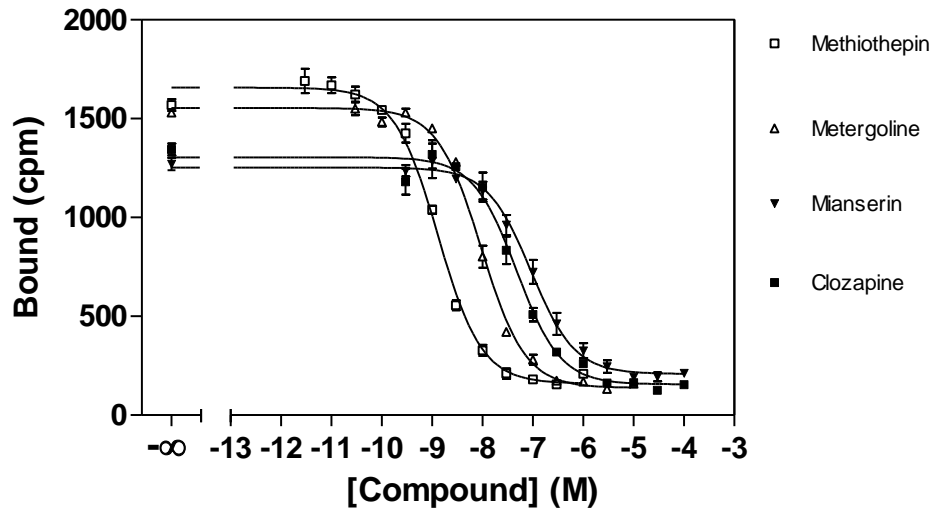


Figure 2: Competition binding assay curve (filtration)
 96-well competition binding assay curve (17 μ g membranes/well, TopCount®). Recommended radioligand concentration = 3 nM.

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

Reference Compounds	Ki (nM)
Methiothepin	0.65
Metergoline	4.7
Mianserin	46
Clozapine	24

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