

human Serotonin 5-HT_{2A} Receptor

Product Number: ES-313-M400UA

Lot Number: 3177493

Material Provided

Membrane: 1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background: CHO-K1

GenBank Accession Number: X57830

Unit Size: 5 µg protein / unit

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.5 mM EDTA, 10 mM 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): 1.1

Expression Level (B_{max}): 5.1 pmol/mg membrane protein.

K_d for [3H]-Ketanserin: 0.15 nM

Protein Concentration: 5 µg/µL

(1) Smith, P.K., et al. (1985). *Anal. Biochem.* 150, 76-85.

Recommended Assay Conditions

Assay Buffer:	50 mM Tris pH 7.4, 4 mM CaCl ₂ , 0.1% ascorbic acid
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: 0.05 mL of membranes + 24.95 mL assay buffer (1:500 dilution)
- 2 - Incubation: 25 µL of incubation buffer or Mianserin (Sigma M2525) 20 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: 60 minutes at 27 °C
- 4 - Filtration: aspirate and wash 9 x 500 µL with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

Lot Specific Data

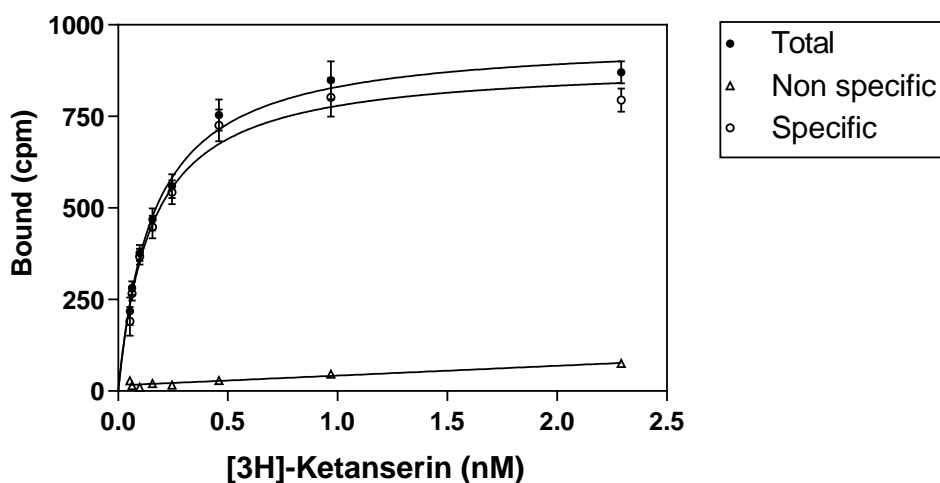


Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (5 µg membranes/well, TopCount®) using [3H]-Ketanserin (Revvity NET1233 Lot No.: 3157271).

Typical Product Data

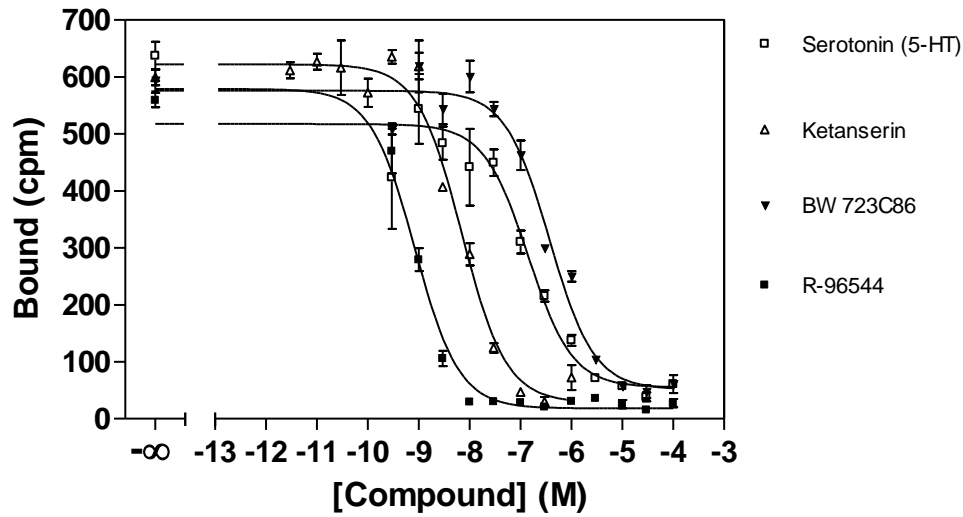


Figure 2: Competition binding assay curve (filtration)
 96-well competition binding assay curve (5 µg membranes/well,
 TopCount®). Recommended radioligand concentration = 1 nM.

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

Reference Compounds	Ki (nM)
Serotonin (5-HT)	62
Ketanserin	3.1
BW 723C86	165
R-96544	0.37

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