

## human Serotonin 5-HT<sub>2B</sub> Receptor

Product No.: ES-314-M400UA

Lot No.: 2969821

### Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: X77307

Unit Size: 7 µg 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): 1.2

Expression Level (B<sub>max</sub>): 3.8 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>3</sup>H]-LSD : 0.3 nM

Protein Concentration: 7 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 <sub>2</sub> , 0.1% ascorbic acid   |
| Wash Buffer:           | 50 mM Tris-HCl pH 7.4  |
| 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 Serotonin (5-HT) (Sigma H9523) 50 $\mu$ 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)<br>500 $\mu$ L of diluted membranes                       |
| 3 - Incubation time:   | 30 minutes at 37 $^{\circ}$ 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

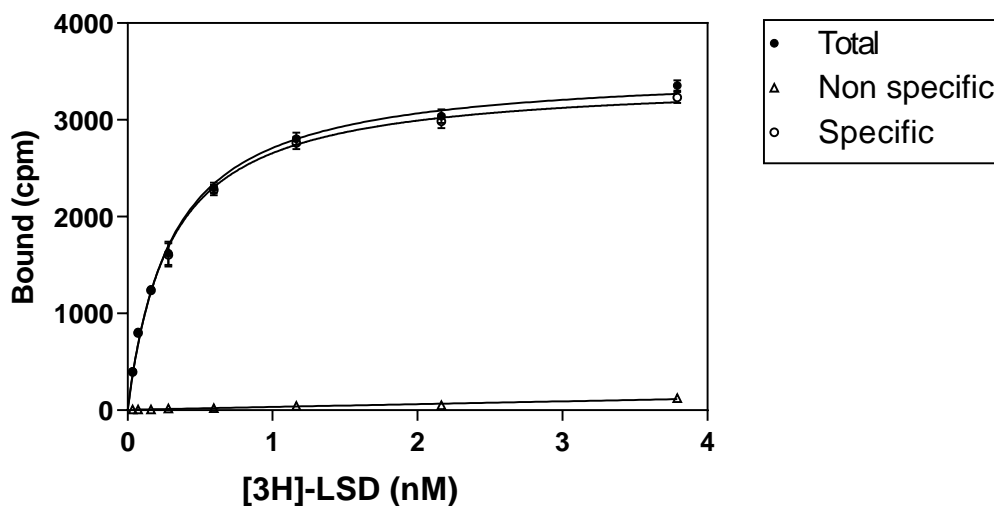


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (7  $\mu$ g membranes/well, TopCount<sup>®</sup>) using [<sup>3</sup>H]-LSD (Revvity NET638 Lot No.: 2958164)

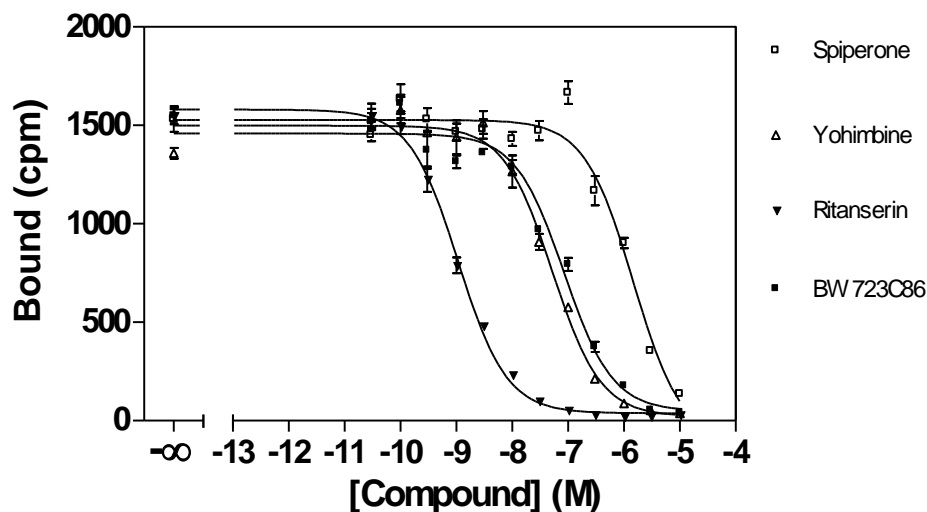


Figure 2: Competition binding assay curve (filtration)  
96-well competition binding assay curve (7  $\mu\text{g}$  membranes/well, TopCount<sup>®</sup>). Recommended radioligand concentration = 1 nM.

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

| Reference Compounds | Ki (nM) |
|---------------------|---------|
| Spiperone           | 813     |
| Yohimbine           | 29      |
| Ritanserin          | 0.60    |
| BW 723C86           | 47      |

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