

## human Serotonin 5-HT<sub>5A</sub> Receptor

Product No.: ES-401-M400UA

Lot No.: 1763491B

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### Material Provided

Membranes: 1 x 400 units / 1000 µL frozen aliquot

### Product Information

Cellular Background: CHO-K1

GenBank Accession Number: AF498985

Unit Size: 15 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): N/A

Expression Level (B<sub>max</sub>): 12.1 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>3</sup>H]-LSD: 0.74 nM

Protein Concentration: 6 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, 0.3% BSA
- 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: 0.125 mL of membranes + 24.875 mL assay buffer (1:200 dilution)
  - 2 - Incubation: 25  $\mu$ L of incubation buffer or 5-Carboxamidotriptamine (5-CT) (Sigma C117) 10  $\mu$ M final for non specific binding (Saturation binding assay)  
*For competition binding assay: 25  $\mu$ L of reference compounds at decreasing concentrations (see figure 2)*  
25  $\mu$ L of radioligand at the appropriate concentration (see graph below)  
500  $\mu$ L of diluted membranes
  - 3 - Incubation time: 60 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 % BSA).

Lot Specific Data

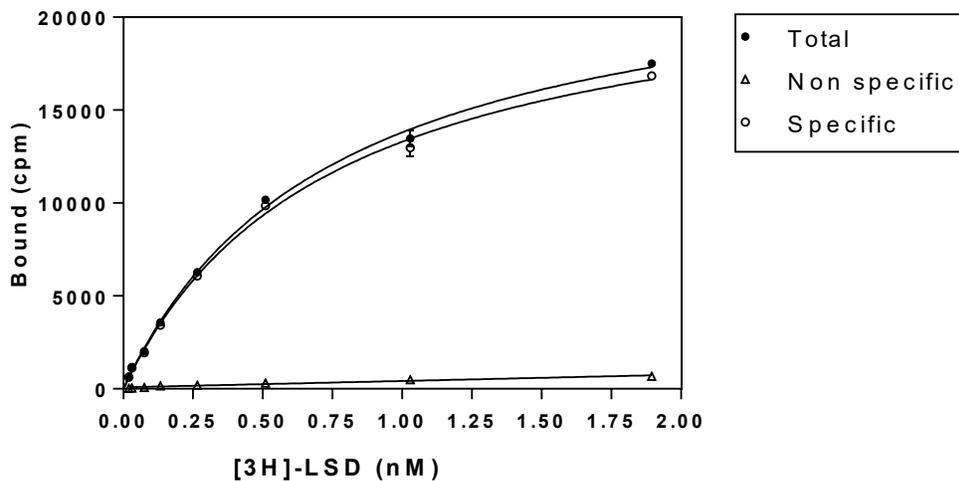


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (15  $\mu$ g membranes/well, TopCount<sup>®</sup>) using [3H]-LSD (Revvity NET638 Lot No.: 1749682)

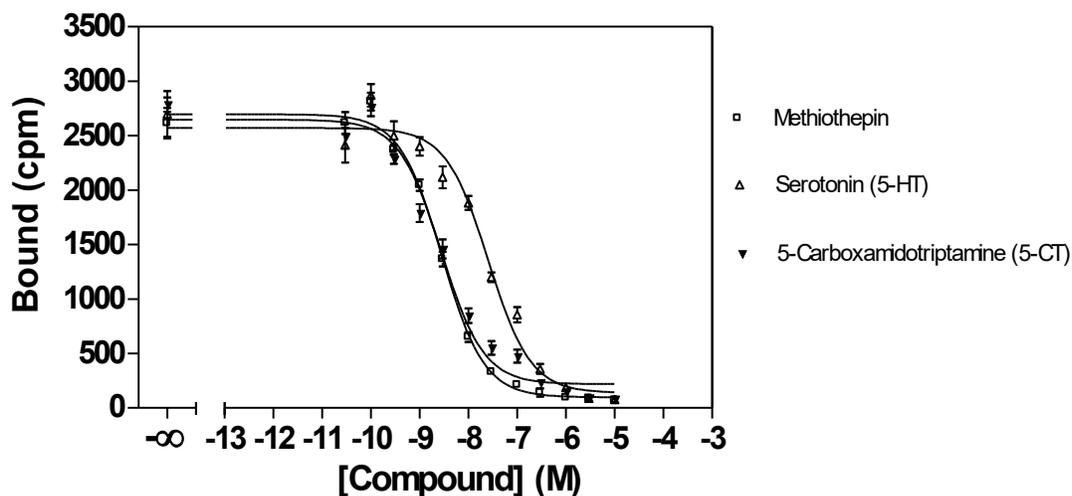


Figure 2: Competition binding assay curve (filtration)  
 96-well competition binding assay curve (15  $\mu$ g membranes/well, TopCount®). Recommended radioligand concentration = 1 nM.

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

| Reference Compounds            | Ki (nM) |
|--------------------------------|---------|
| Methiothepin                   | 1.51    |
| Serotonin (5-HT)               | 13.5    |
| 5-Carboxamidotriptamine (5-CT) | 1.48    |
|                                |         |

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