

## human Serotonin 5-HT<sub>2C(ne)</sub> Receptor

Product No.: 6110548400UA

Lot No.: 3111014

### Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

### Product Information

Cellular Background: HEK293

GenBank Accession Number: M81778 (C23S)

Unit Size: 3.5 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): 1.1

Expression Level (B<sub>max</sub>): 5.9 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>3</sup>H]-Mesulergine: 0.22 nM

Protein Concentration: 3.5 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 MgCl<sub>2</sub>, 1 mM EDTA , 0.1% 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: 0.05 mL of membranes + 24.95 mL assay buffer (1:500 dilution)
- 2 - Incubation: 25  $\mu$ L of incubation buffer or Mianserin (Sigma M2525) 1  $\mu$ 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 °C
- 4 - Filtration: aspirate and wash 9 x 500  $\mu$ L with ice cold wash buffer over GF/C filter (presoaked in 0.3% PEI).

Lot Specific Data

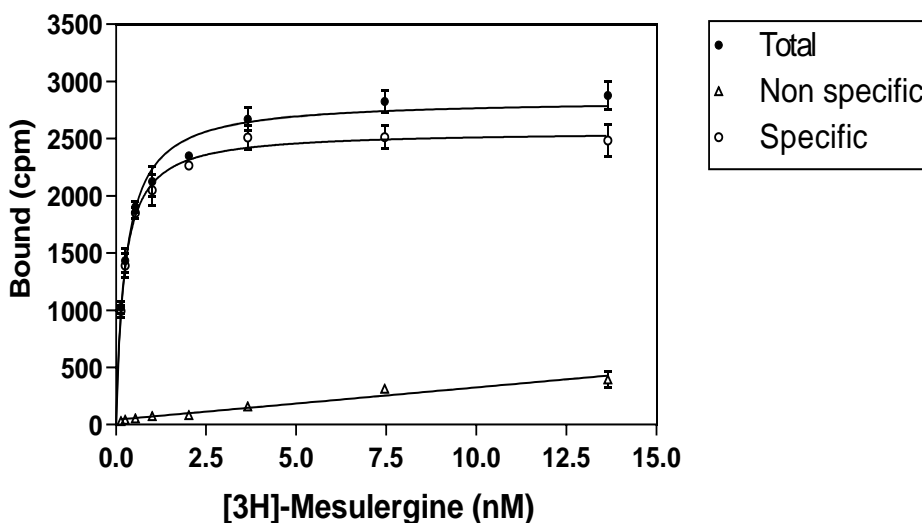


Figure 1: Saturation binding assay curve (filtration)  
 96-well saturation binding assay curve (3.5  $\mu$ g membranes/well, TopCount®) using [<sup>3</sup>H]-Mesulergine (Revvity NET1148 Lot No.: 3114520)

Typical Product Data

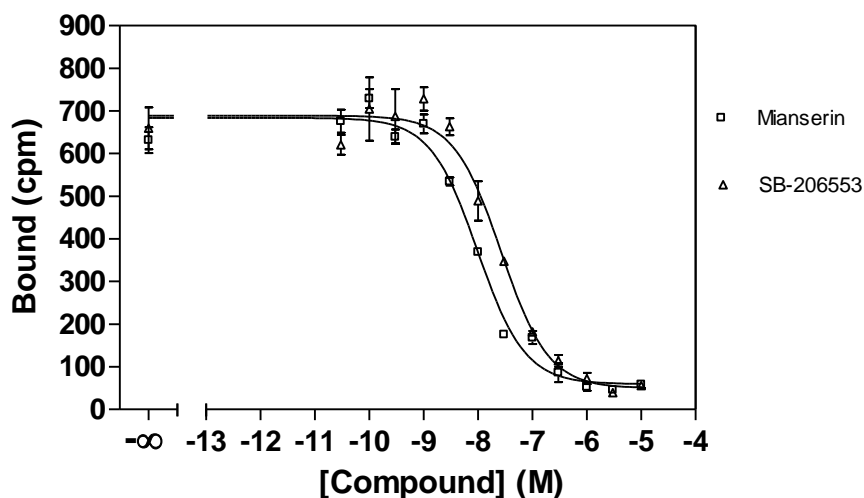


Figure 2: Competition binding assay curve (filtration)  
 96-well competition binding assay curve (3.5 µg membranes/well, TopCount®). Recommended radioligand concentration = 2 nM.

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

Reference Compounds	Ki (nM)
Mianserin	4.9
SB-206553	13.3

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