

## human Opioid NOP (ORL1) Receptor

Product No.: RBHORLM400UA

Lot No.: 2402164

### Material Provided

Membranes: 1 x 400 units / 400  $\mu$ L frozen aliquot

### Product Information

Cellular Background: HEK293

GenBank Accession Number: NM\_000913

Unit Size: 14  $\mu$ g 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

$B_{max}$  and  $K_d$  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 ( $B_{max}$  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): 1.0

Expression Level ( $B_{max}$ ): 1.9 pmol/mg membrane protein.

$K_d$  for [<sup>3</sup>H]-Nociceptin : 0.02 nM

Protein Concentration: 14  $\mu$ g/ $\mu$ L

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

## Recommended Assay Conditions

|                        |  |
|------------------------|--|
| Assay Buffer:          | 50 mM Hepes pH 7.4, 10 mM MgCl <sub>2</sub> , 1 mM EDTA  |
| Wash Buffer:           | 50 mM Hepes pH 7.4, 10 mM MgCl <sub>2</sub> , 1 mM EDTA  |
| 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 Nociceptin (1-13) amide (Bachem H-4072) 1 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)<br>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

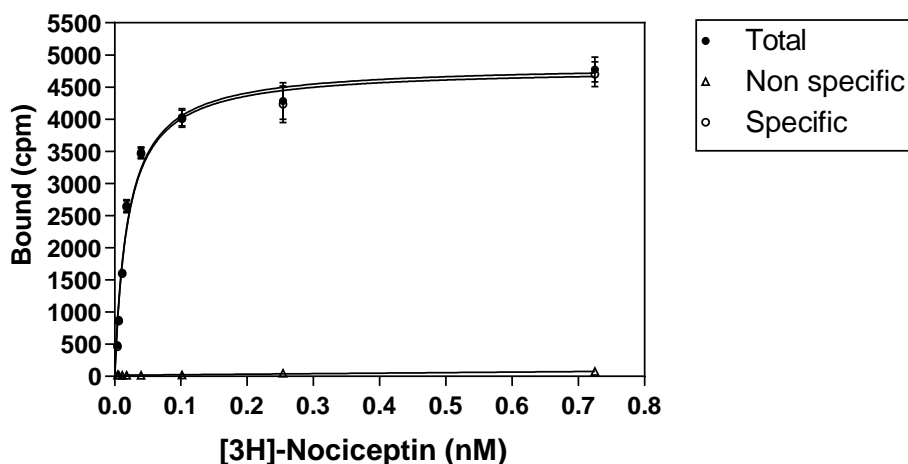


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (14 µg membranes/well, TopCount®) using [<sup>3</sup>H]-Nociceptin (Revvity NET1130 Lot No.: 2403105)

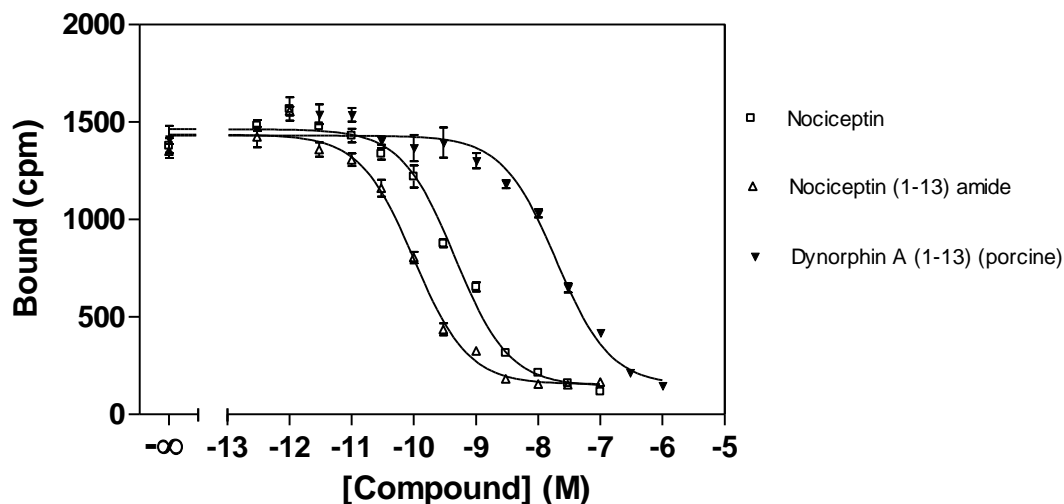


Figure 2: Competition binding assay curve (filtration)  
 96-well competition binding assay curve (14  $\mu$ g membranes/well, TopCount<sup>®</sup>). Recommended radioligand concentration = 0.1 nM.

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

| Reference Compounds          | K <sub>i</sub> (nM) |
|------------------------------|---------------------|
| Nociceptin                   | 0.27                |
| Nociceptin (1-13) amide      | 0.06                |
| Dynorphin A (1-13) (porcine) | 11.2                |
|                              |                     |

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