

## human Neurokinin NK<sub>1</sub> Receptor

Product No.: 6110551400UA

Lot No.: 2596662

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### Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

### Product Information

Cellular Background: UC11

GenBank Accession Number: Endogenous

Unit Size: 10 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): 0.6

Expression Level (B<sub>max</sub>): 0.33 pmol/mg membrane protein.

K<sub>d</sub> for [<sup>125</sup>I]-Substance P: 0.04 nM

Protein Concentration: 10 µg/µL

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

Recommended Assay Conditions

- Assay Buffer: 40 mM Hepes pH 7.4, 5 mM MgCl<sub>2</sub>, 1 mM EDTA, 0.5 % BSA, 0.025% Bacitracin (Sigma B 8800), 25 μM Phosphoramidon
- Wash Buffer: 50 mM Tris-HCl pH 7.4
- Binding Protocol: Binding assays are performed in 200 μL total volume according to the following conditions:
- 1 - Membrane dilution: 0.05 mL of membranes + 7.45 mL assay buffer (1:150 dilution)
  - 2 - Incubation: 25 μL of incubation buffer or L-703,606 (Sigma L119) 10 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)  
150 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 0.3% PEI).

Lot Specific Data

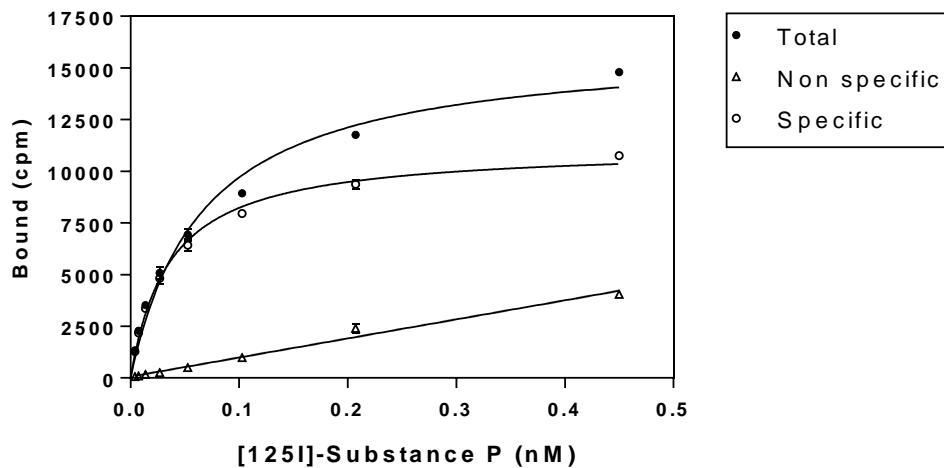


Figure 1: Saturation binding assay curve (filtration)  
96-well saturation binding assay curve (10 μg membranes/well, TopCount®) using [<sup>125</sup>I]-Substance P (Revvity NEX190 Lot No.: CXA0190)

Typical Product Data

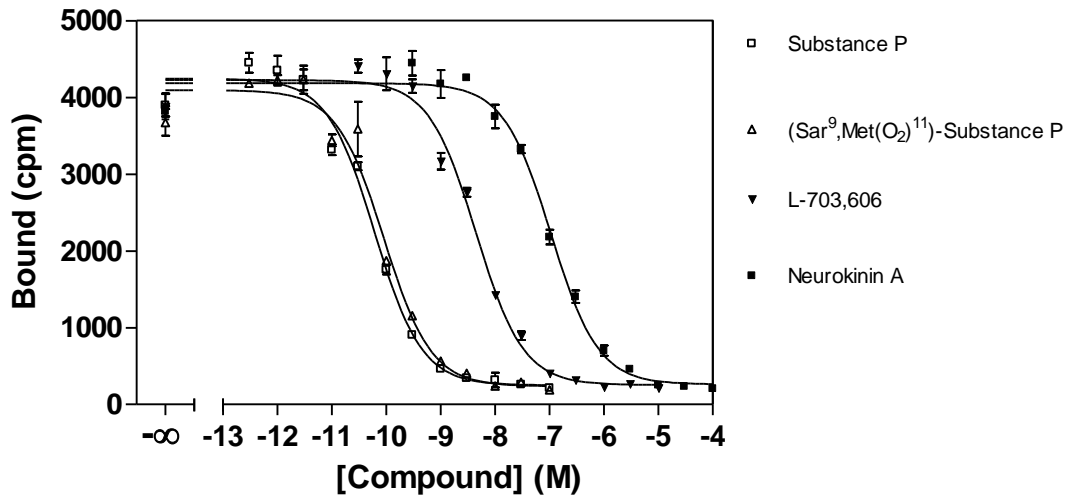


Figure 2: Competition binding assay curve (filtration)  
96-well competition binding assay curve (10 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.15 nM.

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

Reference Compounds	Ki (nM)
Substance P	0.017
(Sar <sup>9</sup> ,Met(O <sub>2</sub> ) <sup>11</sup> )-Substance P	0.025
L-703,606	1.22
Neurokinin A	29.2

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