

Caution: For Laboratory Use. A research reagent for research purposes only

## human Neuropeptide FF NPFF1 Receptor

**Product No.:** ES-491-M400UA

**Lot No.:** 409-145-A

### Material Provided

**Membranes:** 1 x 400 units / 400 µL frozen aliquot

### Product Information

**Cellular Background:** CHO-K1

**GenBank Accession Number:** AF268898

**Unit Size:** 0.5 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):** N/A

**Expression Level (B<sub>MAX</sub>):** 2.2 pmol/mg membrane protein.

**K<sub>D</sub> for [<sup>125</sup>I]-Neuropeptide FF:** 0.37 nM

**Protein Concentration:** 0.5 µg/µL

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

## Recommended Assay Conditions

**Assay Buffer:** 25 mM Hepes pH 7.4, 10 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.5% BSA

**Wash Buffer:** 50 mM Tris-HCl pH 7.4

**Binding Protocol:** Binding assays are performed in 200  $\mu$ L 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  $\mu$ L of incubation buffer or Neuropeptide FF (human) (Phoenix 048-41)  
40  $\mu$ M final for non specific binding (Saturation binding assay)

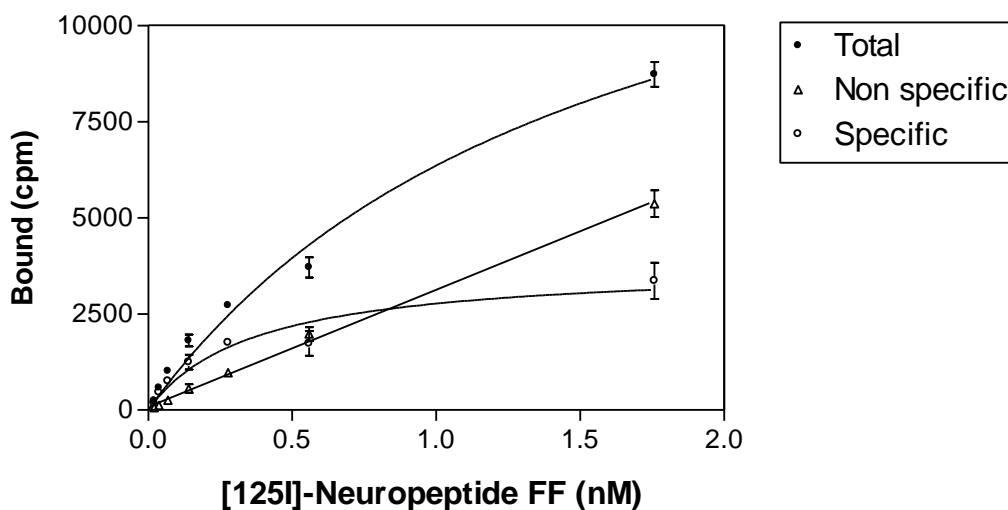
*For competition binding assay: 25  $\mu$ L of reference compounds at decreasing concentrations (see figure 2)*

25  $\mu$ L of radioligand at the appropriate concentration (see graph below)  
150  $\mu$ L of diluted membranes

3 - Incubation time: 30 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).

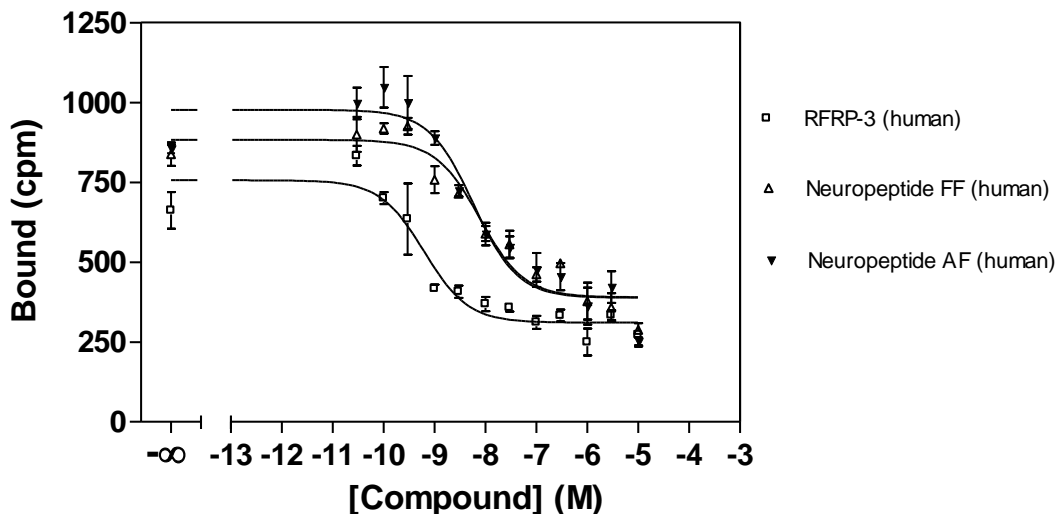
## Lot Specific Data



**Figure 1: Saturation binding assay curve (filtration)**

96-well saturation binding assay curve (0.5  $\mu$ g membranes/well, TopCount<sup>®</sup>) using [<sup>125</sup>I]-Neuropeptide FF (PerkinElmer NEX381 Lot No.: JP81770)

## Typical Product Data



**Figure 2: Competition binding assay curve (filtration)**

96-well competition binding assay curve (0.5  $\mu$ g membranes/well, TopCount®). 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)
RFRP-3 (human)	0.54
Neuropeptide FF (human)	6.8
Neuropeptide AF (human)	5.0

## Suggested Materials and Instrumentation

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