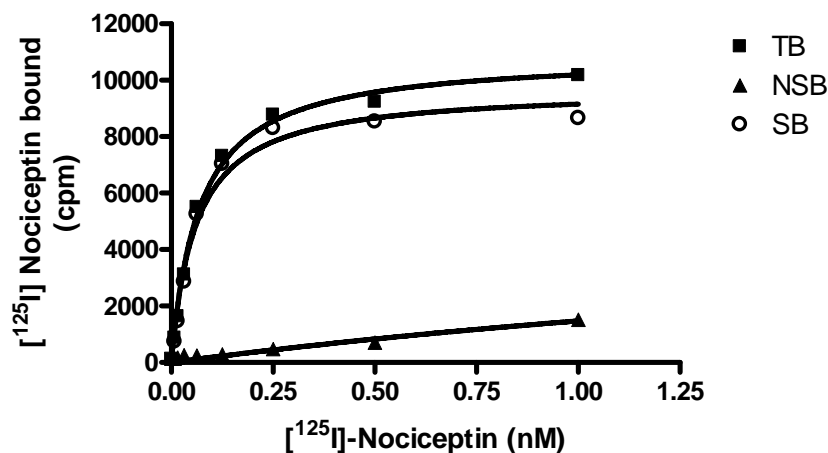


**CHEMISCREEN™ MEMBRANE PREPARATION  
RECOMBINANT HUMAN NOP OPIOID RECEPTOR**

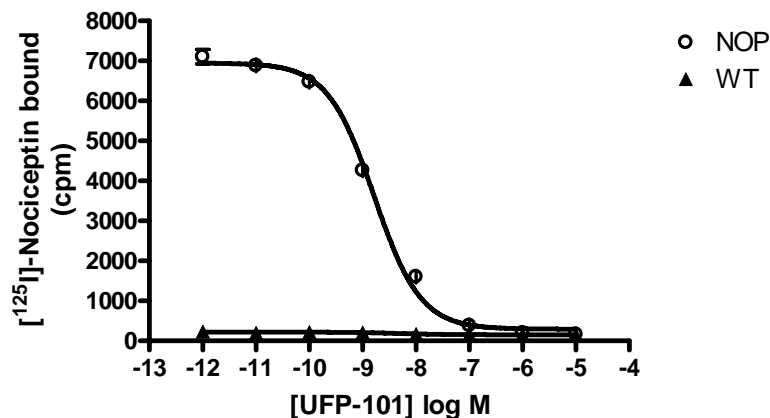
<b>CATALOG NUMBER:</b>	HTS040M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>	RI08010043	<b>VOLUME/CONCENTRATION PER VIAL:</b>	1 mL, 1 mg/mL

**BACKGROUND:** The NOP receptor (also known as ORL1) is related to the opioid receptor family of GPCRs but does not bind to classical opioids. An endogenous ligand for NOP has been characterized and termed orphanin FQ or nociceptin (OFQ/N), which in turn does not bind to other members of the opioid receptor family. NOP is expressed widely in the CNS, and binding of OFQ/N to NOP1 appears to function in nociception, locomotor activity, anxiety, reward, memory and tolerance to classical opioids (Mogil and Pasternak, 2001). Millipore's NOP membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of agonists and antagonists of NOP. The membrane preparations exhibit a  $K_d$  of 0.06 nM for [ $^{125}$ I]-Nociceptin. With 0.1 nM [ $^{125}$ I]-Nociceptin, 5  $\mu$ g/well NOP Membrane Prep typically yields greater than 10-fold signal-to-background ratio.

**APPLICATIONS:** Radioligand binding assay



**Figure 1. Saturation binding for NOP.** 5  $\mu$ g/well NOP Membrane Preparation was incubated with increasing amount of [ $^{125}$ I]-labeled Nociceptin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled UFP-101. Specific binding (SB) was determined by subtracting NSB from TB.



**Figure 2. Competition binding for NOP.** 5  $\mu$ g/well NOP Membrane Preparation and 10  $\mu$ g/well wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.1 nM  $^{125}$ I-labeled Nociceptin and increasing concentrations of unlabeled UFP-101. More than 10-fold signal:background was obtained.

**Table 1.** Signal:background and specific binding values obtained in a competition binding assay with NOP Receptor membrane prep.

	5 $\mu$ g/well
Signal:background	23.8
Specific binding (cpm)	6656

SPECIFICATIONS: 1 unit = 5  $\mu$ g  
 $B_{max}$  for [ $^{125}$ I]-Nociceptin binding: 1.1 pmol/mg protein  
 $K_d$  for [ $^{125}$ I]-Nociceptin binding:  $\sim$ 0.06 nM

TRANSFECTION: Full-length human OPRL1 cDNA encoding NOP (Accession Number: X72304).

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein,  $G_{\alpha 15}$ .

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM Tris-HCl, pH 7.4. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM Tris-HCl, pH 7.4 filtered and stored at 4°C

Radioligand: [ $^{125}$ I]-Nociceptin. (Perkin Elmer#:NEX-324 )

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is

the amount of membrane that will yield greater than 10-fold signal:background with <sup>125</sup>I labeled Nociceptin

**PRESENTATION:**

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.  
Packaging method: Membranes protein were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

**STORAGE/HANDLING:**

Maintain frozen at -70°C up to the expiration date indicated on the product label. Do not freeze and thaw.

**REFERENCES:**

Mogil J.S. and Pasternak G.W. (2001) The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol. Rev.* 53: 381-415.

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