

PRODUCT DATASHEET

ChemiScreen™ NOP Opioid Membrane Preparation

CATALOG NUMBER:	HTS040M	QUANTITY:	200 units
LOT NUMBER:	22G2814	VOLUME/CONCENTRATION:	1 mL, 2 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). 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.

APPLICATIONS: Radioligand binding assay

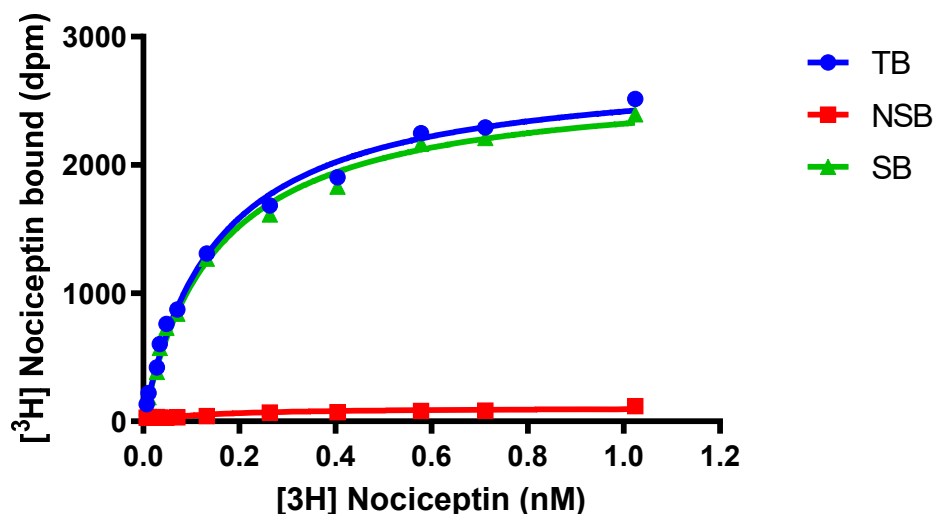


Figure 1. Saturation binding for NOP. 10µg/well NOP Membrane Preparation was incubated with increasing amount of 3H-labeled Nociceptin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of unlabeled Nociceptin at 1 µM. Specific binding (SB) was determined by subtracting NBS from TB. Sample data from a representative lot.

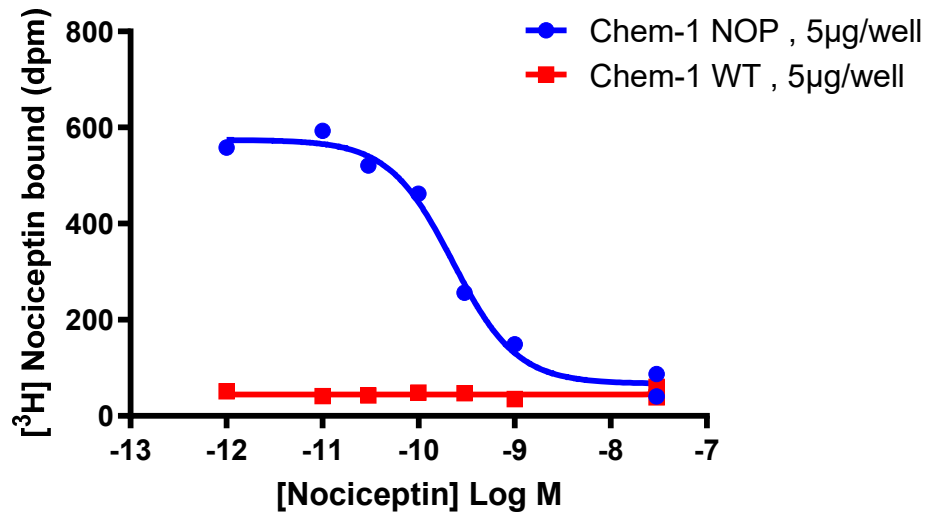


Figure 2. Competition binding for NOP. 10 $\mu\text{g}/\text{well}$ NOP and 5 $\mu\text{g}/\text{well}$ wild type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.06 nM ^3H -labeled Nociceptin and increasing concentrations of unlabeled nociceptin. A 10-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 μg

B_{max} for ^3H -Nociceptin binding: 2696 fmol/mg protein

K_d for ^3H -Nociceptin binding: 0.15 nM

Signal:background: 10-fold

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 1h. Prior to filtration, a GF/B 96-well harvest plate is coated with 50 mM Tris-HCl, pH 7.4 + 0.3% polyethyleneimine. Binding reaction is transferred to the filter plate, and washed 4 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, 5 mM MgCl_2 , 0.1 g/L Bacitracine, 20 mg/L Aprotinine, 0.1% BSA stored at 4°C.

Radioligand: ^3H -Nociceptin. (Perkin Elmer#:NET1130)

Wash Buffer: 50 mM Tris-HCl, pH 7.4.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield a 10-fold signal:background with ^3H labeled Nociceptin.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at -70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

1. 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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