

PRODUCT DATASHEET

 ChemiScreen™ NPBW₁ Neuropeptide B/W Membrane Preparation

CATALOG NUMBER:	HTS180M	QUANTITY:	200 units
LOT NUMBER:	R0708E0013	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: Neuropeptide B (NPB) and neuropeptide W (NPW) are members of a recently identified neuropeptide family that are ligands for two highly similar receptors NPBW₁ and NPBW₂, both of which are coupled to Gi/o protein to inhibit intracellular cAMP production. Highest expression of NPBW₁ mRNA and protein was identified in the amygdala and hypothalamic nuclei. Physiological studies demonstrate that intracerebroventricular infusion of NPBW₁ ligands modulates feeding behavior, regulates the release of corticosterone, prolactin and growth hormone while also modulating pain pathway (Singh and Davenport, 2006). NPBW₁ knock out male mice have shown mild adult-onset obesity and decreased locomotor activity. They become progressively hyperglycaemic and hyperinsulinaemic (Ishii *et al.*, 2003). NPBW₁ 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 antagonists of NPBW₁ receptor interactions with its ligand. The membrane preparations exhibit a K_d of 0.53nM for [¹²⁵I]-Neuropeptide W23. With 5 ug/well NPBW₁ Membrane Prep and 0.35nM [¹²⁵I]-Neuropeptide W23, a greater than 10-fold signal-to-background ratio was obtained

APPLICATIONS: Radioligand binding assay, and GTPγS binding

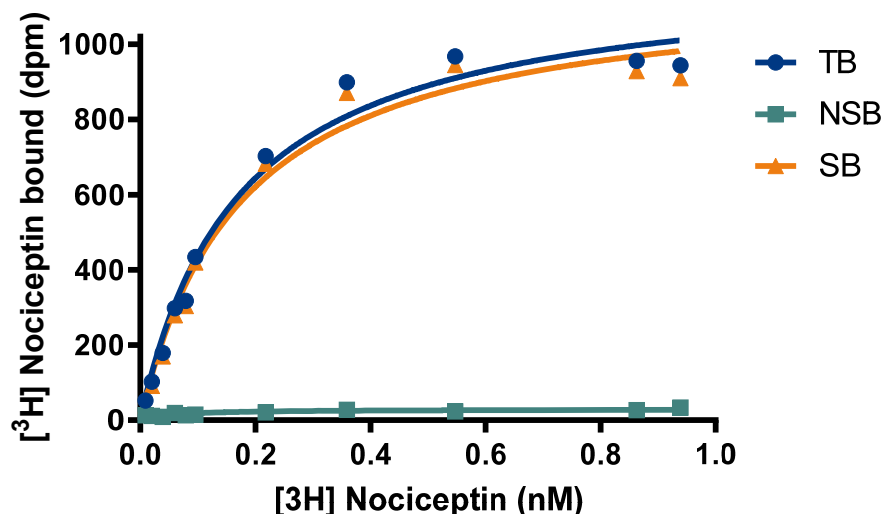


Figure 1. Saturation binding for NPBW₁ Receptor. 5 μg/well NPBW₁ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-Neuropeptide W23 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled human recombinant neuropeptide W30. Specific binding (SB) was determined by subtracting NSB from TB.

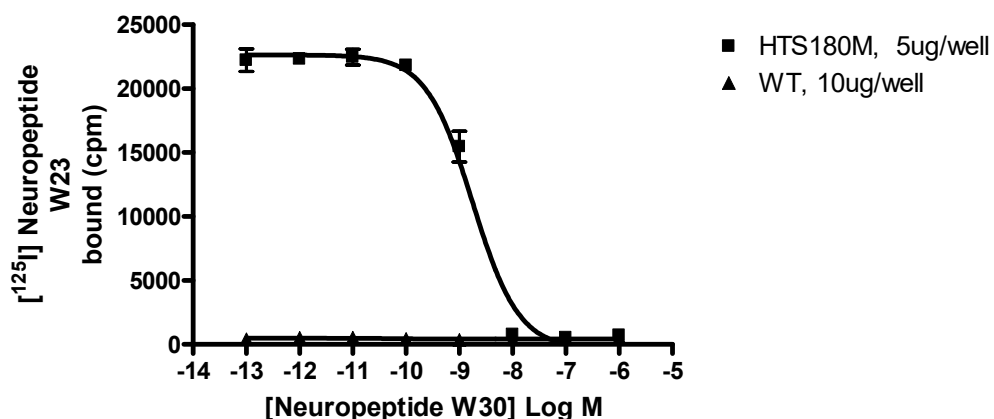


Figure 2. Competition binding for NPBW₁ Receptor. NPBW₁ Receptor Membrane Preparation (5□g/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.35nM [¹²⁵I]-Neuropeptide W23 and increasing concentrations of unlabeled neuropeptide W30, and more than 10- fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [³H]-Nociceptin binding: 8.74pmol/mg
 K_d for [³H]-Nociceptin binding: 0.53 nM
 Signal:background: >10-fold

TRANSFECTION: Full length human GPR7 cDNA encoding NPBW₁ (Accession number NM_005285)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous NPBW₁ Receptor expression.

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 50mM Tris, 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 Hepes, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, filtered and stored at 4°C.

Radioligand: [¹²⁵I]-neuropeptide W23 (Perkin Elmer#: NET-403)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 10-fold signal:background with ¹²⁵I-labeled neuropeptide W23 at 0.35nM.

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. Singh G and Davenport AP (2006) Neuropeptide B and W: neurotransmitters in an emerging G-protein-coupled receptor system. *Br. J. Pharmacol.* 148:1033-41.
2. Ishii M *et al.* (2003). Targeted disruption of GPR7, the endogenous receptor for neuropeptides B and W, leads to metabolic defects and adult-onset obesity. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10540–10545.

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