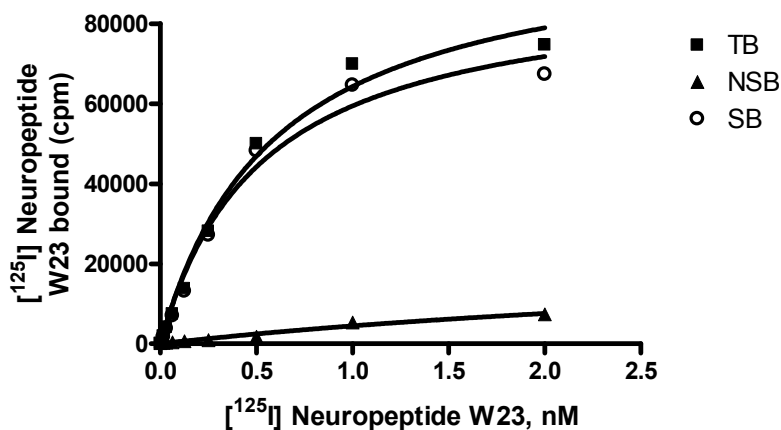


## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN NPBW<sub>1</sub> NEUROPEPTIDE B/W RECEPTOR

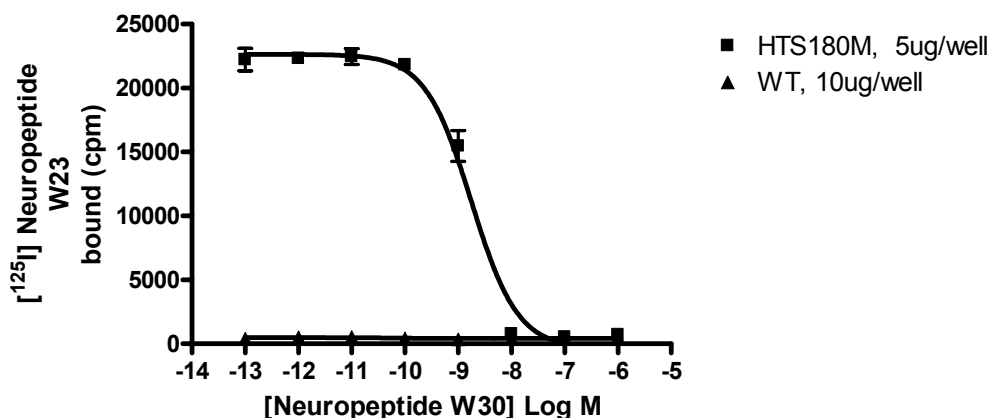
<b>CATALOG NUMBER:</b>	HTS180M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>		<b>VOLUME/CONCENTRATION:</b>	1 mL, 1.0 mg/mL

**BACKGROUND:** Neuropeptide B (NPB) and neuropeptide W (NPW) are members of a recently identified neuropeptide family that are ligands for two highly similar receptors NPBW<sub>1</sub> and NPBW<sub>2</sub>, both of which are coupled to Gi/o protein to inhibit intracellular cAMP production. Highest expression of NPBW<sub>1</sub> mRNA and protein was identified in the amygdala and hypothalamic nuclei. Physiological studies demonstrate that intracerebroventricular infusion of NPBW<sub>1</sub> ligands modulates feeding behavior, regulates the release of corticosterone, prolactin and growth hormone while also modulating pain pathway (Singh and Davenport, 2006). NPBW<sub>1</sub> knock out male mice have shown mild adult-onset obesity and decreased locomotor activity. They become progressively hyperglycaemic and hyperinsulinaemic (Ishii *et al.*, 2003). Millipore's NPBW<sub>1</sub> 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 NPBW<sub>1</sub> receptor interactions with its ligand. The membrane preparations exhibit a K<sub>d</sub> of 0.53nM for [<sup>125</sup>I]-Neuropeptide W23. With 5 ug/well NPBW<sub>1</sub> Membrane Prep and 0.35nM [<sup>125</sup>I]-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 NPBW<sub>1</sub> Receptor.** 5 μg/well NPBW<sub>1</sub> Membrane Preparation was incubated with increasing amount of [<sup>125</sup>I]-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 NPBW<sub>1</sub> Receptor.** NPBW<sub>1</sub> Receptor Membrane Preparation (5µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.35nM [<sup>125</sup>I]-Neuropeptide W23 and increasing concentrations of unlabeled neuropeptide W30, and more than 10- fold signal:background was obtained.

**Table 1.** Signal:background and specific binding values obtained in a competition binding assay with 5 µg/well of NPBW<sub>1</sub> Receptor membrane prep.

	5 µg/well
Signal:background	31.8
Specific binding (cpm)	21932

SPECIFICATIONS: 1 unit = 5 µg membrane preparation  
 Bmax: 8.74pmol/mg  
 K<sub>d</sub>: 0.53 nM

Species: Full length human GPR7 cDNA encoding NPBW<sub>1</sub> (Accession number NM\_005285)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous NPBW<sub>1</sub> 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 MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, filtered and stored at 4°C.

Radioligand: [<sup>125</sup>I]-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 <sup>125</sup>I-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: Membranes protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

**STORAGE/HANDLING:**

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

**REFERENCES:**

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.

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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