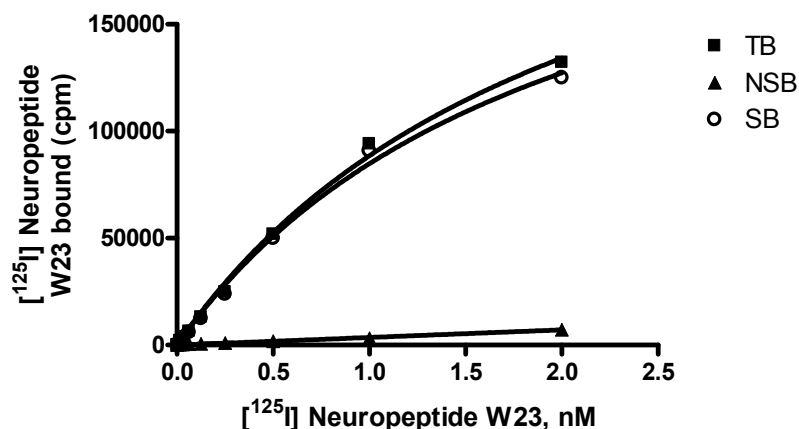


## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN NPBW<sub>2</sub> NEUROPEPTIDE B/W RECEPTOR

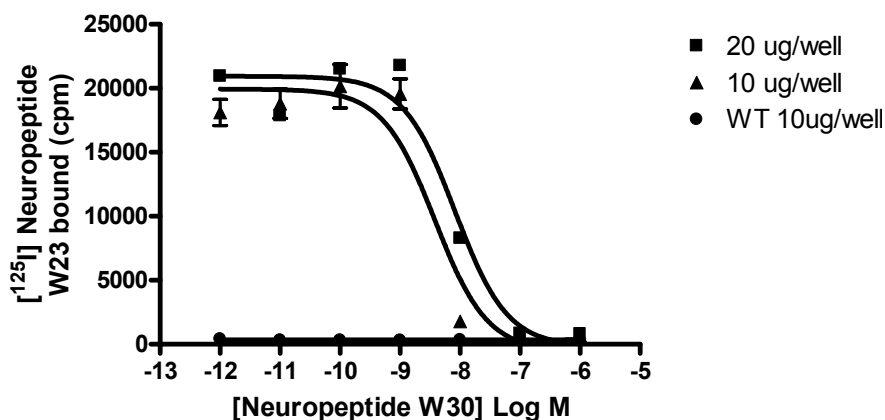
<b>CATALOG NUMBER:</b>	HTS181M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>		<b>VOLUME/CONCENTRATION:</b>	2 mL, 1.0 mg/mL

**BACKGROUND:** Neuropeptide B (NPB) and neuropeptide W (NPW) are members of a recently identified neuropeptide family that are ligands for NPBW<sub>1</sub> and NPBW<sub>2</sub> receptors, both of which are coupled to Gi/o protein to inhibit intracellular cAMP production and share 64% sequence homology (Singh and Davenport, 2006). NPBW<sub>1</sub> recognizes both NPB and NPW with similar nanomolar affinities (with a slight preference for NPB), whereas NPBW<sub>2</sub> is moderately selective for NPW (Tanaka *et al.*, 2003). NPBW<sub>2</sub> is one of a few GPCRs that have no rat or mouse orthologue, although gene encoding NPBW<sub>2</sub> has been discovered in other mammalian species such as rabbit (Lee *et al.*, 1999). NPBW<sub>2</sub> mRNA is known to be expressed in the frontal cortex, parietal cortex hippocampus, caudate nucleus, thalamus, pituitary, adrenal gland, and lymph node (Brezillon *et al.*, 2003). Functions of NPBW<sub>2</sub> may be involved in feeding, weight regulation, and pain response through direct or indirect actions in the central nervous system. Millipore's NPBW<sub>2</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>2</sub> receptor interactions with its ligand. The membrane preparations exhibit a K<sub>d</sub> of 2nM for [<sup>125</sup>I]-Neuropeptide W23. With 10 ug/well NPBW<sub>2</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>2</sub> Receptor.** 5 µg/well NPBW<sub>2</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>2</sub> Receptor.** NPBW<sub>2</sub> Receptor Membrane Preparation (10 and 20  $\mu$ g/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 10 or 20  $\mu$ g/well of NPBW<sub>2</sub> Receptor membrane prep.

	20 $\mu$ g/well	10 $\mu$ g/well
Signal:background	26.3	30.2
Specific binding (cpm)	20148	19282

SPECIFICATIONS: 1 unit = 10.0  $\mu$ g membrane preparation  
 Bmax: 11.34 pmol/mg  
 K<sub>d</sub>: 2 nM

Species: Full length human GPR8 cDNA encoding NPBW2 (Accession number NM\_005286)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous NPBW<sub>2</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:**

Tanaka H *et al.* (2003) Characterization of a family of endogenous neuropeptide ligands for the G protein-coupled receptors GPR7 and GPR8. *Proc. Natl. Acad. Sci. USA* 100: 6251–6256.

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.

Brezillon S *et al.* (2003). Identification of natural ligands for the orphan G protein-coupled receptors GPR7 and GPR8. *J. Biol. Chem.* 278: 776–783.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

(c) 2007: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.