eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ NPBW$_2$ Neuropeptide B/W Membrane Preparation

CATALOG NUMBER:	HTS181M	**QUANTITY:**	200 units
LOT NUMBER:	R0708E0014	**VOLUME/CONCENTRATION:**	2 mL, 1 mg/mL

BACKGROUND:

Neuropeptide B (NPB) and neuropeptide W (NPW) are members of a recently identified neuropeptide family that are ligands for NPBW$_1$ and NPBW$_2$ 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$_1$ recognizes both NPB and NPW with similar nanomolar affinities (with a slight preference for NPB), whereas NPBW$_2$ is moderately selective for NPW (Tanaka *et al.*, 2003). NPBW$_2$ is one of a few GPCRs that have no rat or mouse orthologue, although gene encoding NPBW$_2$ has been discovered in other mammalian species such as rabbit (Lee *et al.*, 1999). NPBW$_2$ 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$_2$ may be involved in feeding, weight regulation, and pain response through direct or indirect actions in the central nervous system. NPBW$_2$ 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$_2$ receptor interactions with its ligand. The membrane preparations exhibit a Kd of 2nM for [^{125}I]-Neuropeptide W23. With 10 ug/well NPBW$_2$ Membrane Prep and 0.35nM [^{125}I]-Neuropeptide W23, a greater than 10-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

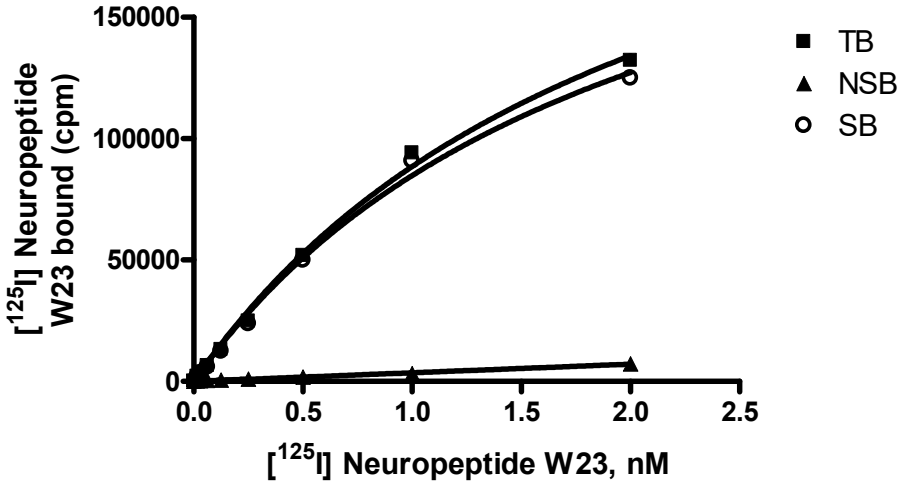

Figure 1. Saturation binding for NPBW$_2$ Receptor. 5 μg/well NPBW$_2$ Membrane Preparation was incubated with increasing amount of [^{125}I]-Neuropeptide W23 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled human recombinant neuropeptide W30. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

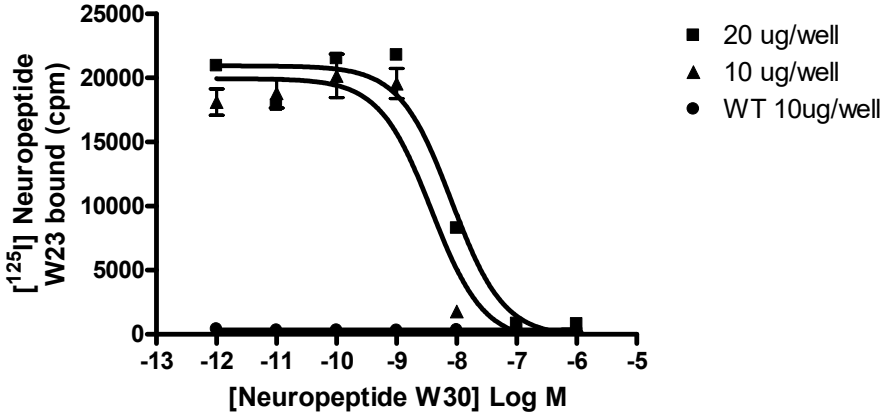

Figure 2. Competition binding for NPBW$_2$ Receptor. NPBW$_2$ Receptor Membrane Preparation (10 and 20 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.35nM [^{125}I]-Neuropeptide W23 and increasing concentrations of unlabeled neuropeptide W30, and more than 10-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg
Bmax: 11.34 pmol/mg
K_d: 2 nM
Signal:background: >10-fold

TRANSFECTION: Full length human GPR8 cDNA encoding NPBW2 (Accession number NM_005286)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous NPBW$_2$ 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 $MgCl_2$, 1 mM $CaCl_2$, filtered and stored at 4°C.

Radioligand: [^{125}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 ^{125}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: 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. 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.

2. 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.

3. 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.