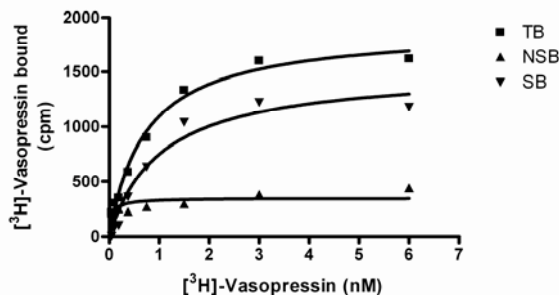


## CHEMISCREEN<sup>™</sup> MEMBRANE PREPARATION RECOMBINANT HUMAN V<sub>1B</sub> VASOPRESSIN RECEPTOR

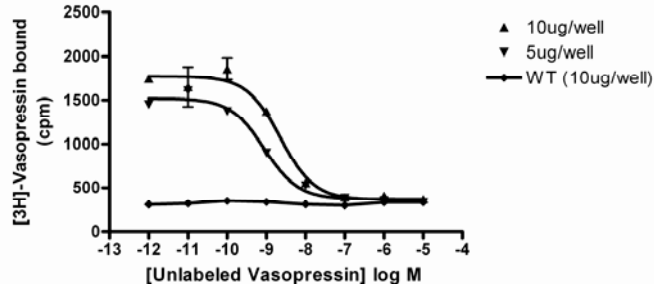
**CATALOG NUMBER:** HTS136M      **QUANTITY:** 200 units  
**LOT NUMBER:**      **VOLUME/CONCENTRATION:** 2 mL, 1 mg/mL

**BACKGROUND:** Arginine vasopressin (AVP) is a 9 amino acid peptide that functions as an antidiuretic, vasoconstrictor and neurotransmitter. The three vasopressin receptors, V<sub>1A</sub>, V<sub>1B</sub> and V<sub>2</sub>, are GPCRs; V<sub>1A</sub> and V<sub>1B</sub> couple to G<sub>q</sub> and calcium release, whereas V<sub>2</sub> couples to G<sub>s</sub> (Bimbaumer, 2000). The V<sub>1B</sub> receptor is expressed prominently in the anterior pituitary, where it mediates vasopressin-induced release of ACTH (Tanoue *et al.*, 2004). A selective antagonist of V<sub>1B</sub> has recently been developed and shown to reduce depression, anxiety, and aggression in rodents (Blanchard *et al.*, 2005; Griebel *et al.*, 2002). Chemicon's cloned human V<sub>1B</sub>-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant V<sub>1B</sub> expression on the cell surface and contains high levels of the promiscuous G protein G $\alpha$ 15 to couple the receptor to the calcium signaling pathway. Chemicon's V<sub>1B</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 V<sub>1B</sub> interactions with vasopressin. The membrane preparations exhibit a K<sub>d</sub> of 1.03 nM for [<sup>3</sup>H]-vasopressin. With 10  $\mu$ g/well V<sub>1B</sub> Membrane Prep and 1.5 nM [<sup>3</sup>H]-vasopressin, a greater than 4-fold signal-to-background ratio was obtained.

**APPLICATIONS:** Radioligand binding assay, and GTP $\gamma$ S binding.



**Figure 1. Saturation binding for V<sub>1B</sub>.** 5  $\mu$ g/well V<sub>1B</sub> Membrane Preparation was incubated with increasing amount of [<sup>3</sup>H]-vasopressin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled vasopressin. Specific binding (SB) was determined by subtracting NSB from TB.



**Figure 2. Competition binding for V<sub>1B</sub>.** V<sub>1B</sub> Membrane Preparation (5 or 10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 1.5 nM [<sup>3</sup>H]-vasopressin and increasing concentrations of unlabeled vasopressin, and more than 4- fold signal:background was obtained.

**Table 1.** Signal:background and specific binding values obtained in a competition binding assay with varying amounts of V<sub>1B</sub> membrane prep.

	10 µg/well	5 µg/well
Signal:background	4.93	4.15
Specific binding (cpm)	1774	1590

SPECIFICATIONS: 1 unit = 10 µg membrane preparation  
B<sub>max</sub>: 2.79 pmol/mg  
K<sub>d</sub>: 1.03 nM

Species: Human V<sub>1B</sub> (Accession number NM\_000707)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous V<sub>1B</sub> 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, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. 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>, 0.2% BSA, filtered and stored at 4°C

Radioligand: [<sup>3</sup>H] vasopressin (Perkin Elmer # NET800)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, 0.1% BSA, 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 4-fold signal:background with <sup>3</sup>H-labeled vasopressin at 1.5 nM.

- PRESENTATION:** Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.  
Packaging method: Membranes protein were 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:** Birnbaumer M (2000) Vasopressin receptors. *Trends Endocrinol. Metab.* 11:406-10.
- Blanchard RJ *et al.* (2005) AVP V1b selective antagonist SSR149415 blocks aggressive behaviors in hamsters. *Pharmacol. Biochem. Behav.* 80: 189-194.
- Griebel G *et al.* (2002) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V<sub>1b</sub> receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc. Natl. Acad. Sci. USA* 99: 6370-6375.
- Tanoue A *et al.* (2004) The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. *J. Clin. Invest.* 113: 302-309.

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.

**Important Note:** *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

© 2002-2004 CHEMICON® International, Inc. - By CHEMICON® International, Inc. All rights reserved. No part of these works may be reproduced in any form without permissions in writing.