

PRODUCT DATASHEET
ChemiScreen™ VPAC₂ VIP/PACAP Membrane Preparation

CATALOG NUMBER:	HTS060M	QUANTITY:	200 units
LOT NUMBER:	1966642	VOLUME/CONCENTRATION:	4mL, 0.5 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_{1a}, V_{1b} and V₂, are GPCRs; V_{1a} and V_{1b} couple to G_q and calcium release, whereas V₂ couples to G_s. V₂ expressed in renal collecting ducts plays an important role in regulating renal free water excretion (Birnbaumer, 2000). Mutations in V₂ result in X-linked nephrogenic diabetes insipidus, a syndrome in which the kidney is unable to concentrate urine, leading to dehydration and hypernatremia (Birnbaumer, 2001). Conversely, elevated levels of AVP lead to hyponatremia in the syndrome of inappropriate antidiuretic hormone secretion (SIADH), congestive heart failure or cirrhosis, and V₂ selective antagonists have been developed to treat these conditions (Verbalis, 2002). V₂ 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 V₂ interactions and its ligands. The membrane preparations exhibit a K_d of 2.83 nM for [³H]-[Arg⁸] Vasopressin. With 3.5 nM [³H]-[Arg⁸] Vasopressin, 10 µg/well V₂ Membrane Prep yields greater than 8-fold signal-to-background ratio.

APPLICATIONS: Radioligand Binding Assay

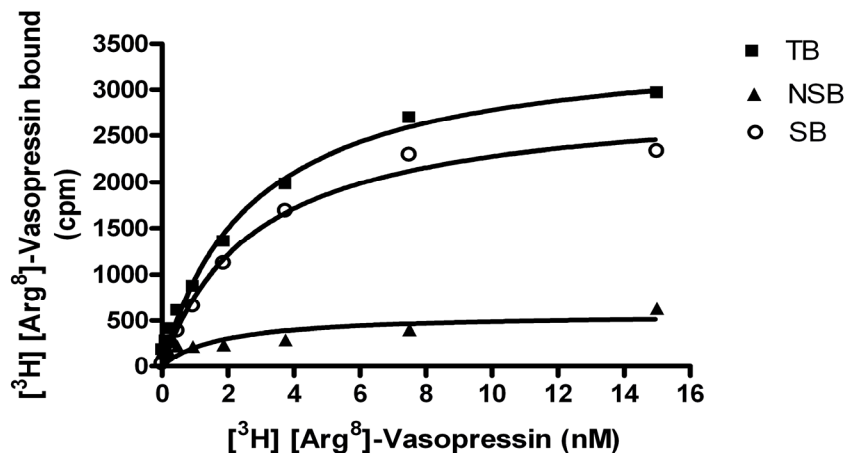


Figure 1. Saturation Binding for V₂. 5 µg/well V₂ Membrane Preparation was incubated with increasing amount of ³H labeled [Arg⁸] Vasopressin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled [Arg⁸] Vasopressin. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

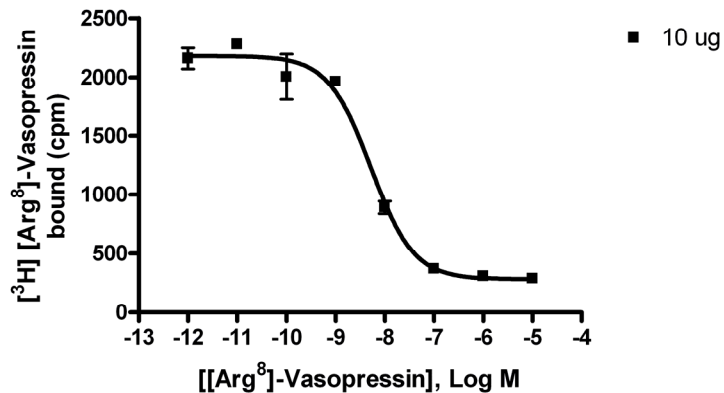


Figure 2. Competition Binding for V₂. V₂ Membrane Preparation (10 µg/well in a 96-well plate) was incubated with 3.5 nM ³H labeled [Arg⁸] Vasopressin and increasing concentrations of unlabeled [Arg⁸] Vasopressin, and subjected to filtration binding. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [³H]-[Arg⁸] Vasopressin binding: 18.3 pmol/mg protein
 K_d for [³H]-[Arg⁸] Vasopressin binding: ~ 2.83 nM
 Signal:Background: ≥8-fold

Species: Human VPAC₂ (Accession number NM_000054)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous V₂ 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [³H]-[Arg⁸] Vasopressin (Perkin Elmer#:NET-800)

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 a unit is the amount of membrane that will yield greater than 8-fold signal:background with ³H labeled [Arg⁸] Vasopressin at 3.5 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 0.5 mg/ml 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Birnbaumer M (2000) Vasopressin receptors. *Trends Endocrinol. Metab.* 11:406-10
2. Birnbaumer M (2001) The V₂ vasopressin receptor mutations and fluid homeostasis. *Cardiovasc. Res.* 51: 409-415.
3. Verbalis JG (2002) Vasopressin V₂ receptor antagonists. *J. Mol. Endocrinol.* 29: 1-9.

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