

PRODUCT DATASHEET
ChemiScreen™ V_{1A} VASOPRESSIN RECEPTOR Membrane Preparation

CATALOG NUMBER:	HTS059M	QUANTITY:	200 units
LOT NUMBER:	21G0105	VOLUME/CONCENTRATION:	1 mL, 2 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. V₁ receptors regulate fluid homeostasis by stimulating contraction of glomerular mesangial cells and renal vascular smooth muscle cells (Birnbaumer, 2000). V_{1A} 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 V_{1A} interactions with its ligands. The membrane preparations exhibit a K_d of 55 pM for [¹²⁵I]-vasopressin antagonist ([¹²⁵I]-phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH₂). With 10 µg/well V_{1A} Membrane Prep and 0.1 nM [¹²⁵I]-vasopressin V_{1A} antagonist, greater 12-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

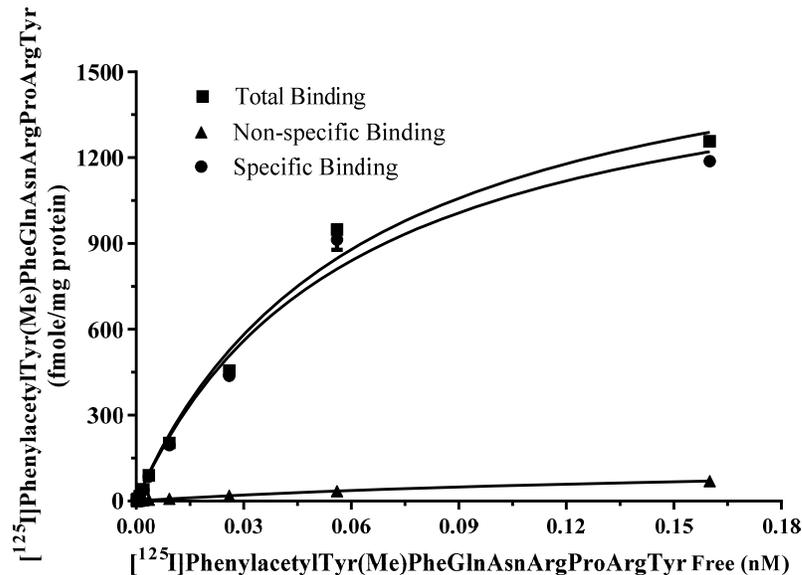


Figure 1. Saturation binding for V_{1A}. 10 µg/well V_{1A} Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled vasopressin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled [Arg⁸]-Vasopressin. Specific binding (SB) was determined by subtracting NSB from TB.

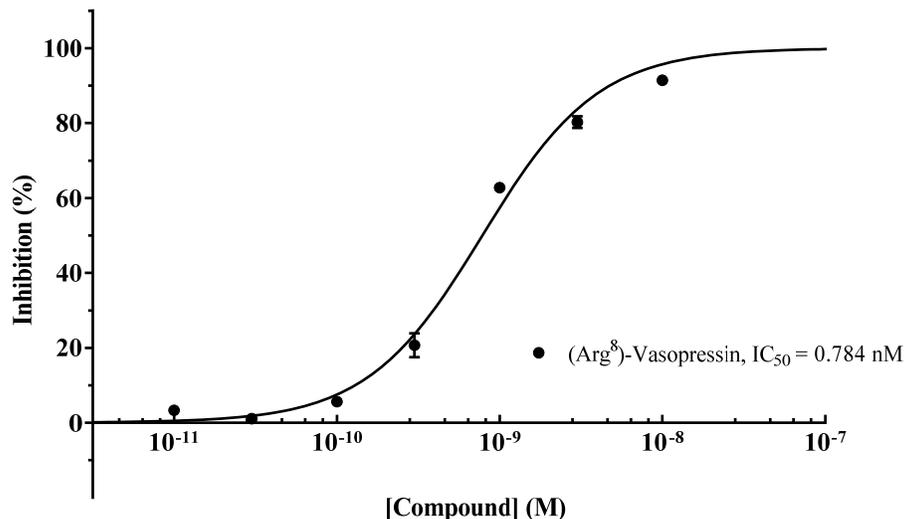


Figure 2. Competition binding for V_{1A}. 2 µg/well V_{1A} Membrane Preparation (HTS059M) and Wild-Type Chem-1 Membrane Preparation (HTS000MC1) were incubated with 0.03 nM ¹²⁵I-labeled vasopressin V_{1A} antagonist and increasing concentrations of unlabeled [Arg⁸]-Vasopressin.

SPECIFICATIONS: 1 unit = 10 µg
 B_{max}: 1.68 pmol/mg protein
 K_d: 60 pM

TRANSFECTION: Human AVPR1 cDNA encoding V_{1A} (Accession number NM_000706)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous V_{1A} 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 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: Radioligand: [¹²⁵I] vasopressin V_{1A} antagonist (Perkin Elmer catalog # NEX310)

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 12-fold signal:background with ¹²⁵I-labeled vasopressin V_{1A} antagonist at 0.1 nM

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: 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: Birnbaumer M (2000) Vasopressin receptors. Trends Endocrinol. Metab. 11:406-10

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