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PRODUCT DATASHEET

ChemiScreen™ V_{1B} Vasopressin Membrane Preparation

CATALOG NUMBER: HTS136M

QUANTITY: 200 units

LOT NUMBER:

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 V_2, are GPCRs; V_{1A} and V_{1B} couple to G_q and calcium release, whereas V_2 couples to G_s (Birnbaumer, 2000). The V_{1B} 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_{1B} has recently been developed and shown to reduce depression, anxiety, and aggression in rodents (Blanchard *et al*, 2005; Griebel *et al.*, 2002). Cloned human V_{1B}-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant V_{1B} expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. V_{1B} 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_{1B} interactions with vasopressin. The membrane preparations exhibit a K_d of 1.03 nM for [^{3}H]-vasopressin. With 10 µg/well V_{1B} Membrane Prep and 1.5 nM [^{3}H]-vasopressin, a greater than 4-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

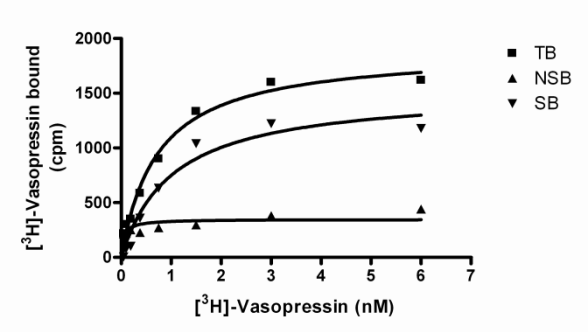

Figure 1. Saturation binding for V_{1B}. 5 µg/well V_{1B} Membrane Preparation was incubated with increasing amount of [^{3}H]-vasopressin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled vasopressin. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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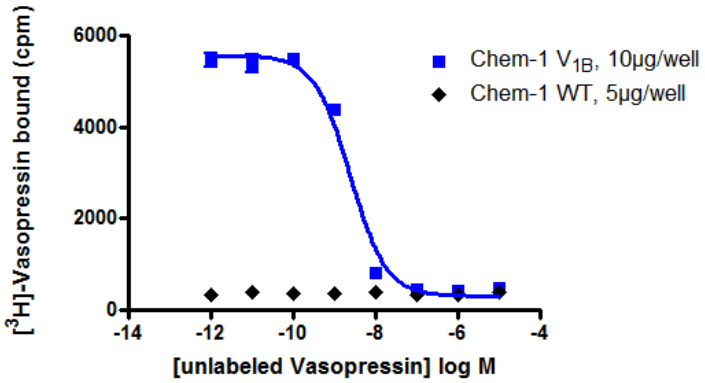

Figure 2. Competition binding for V_{1B}. V_{1B} Membrane Preparation (10 ·g/well) or Wild-Type Chem-1 membrane preparation (5 µg/well; Catalog # HTS000MC1) was incubated with 1.5 nM [^{3}H]-vasopressin and increasing concentrations of unlabeled vasopressin, and more than 4-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
B_{max}: 2.79 pmol/mg
K_d: 1.03 nM
Signal:background: ≥4-fold

Species: Human V_{1B} (Accession number NM_000707)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous V_{1B} 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{3}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 a unit is the amount of membrane that will yield greater than 4-fold signal:background with ^{3}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: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80^{0}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. Birnbaumer M (2000) Vasopressin receptors. *Trends Endocrinol. Metab.* 11:406-10.

2. Blanchard RJ *et al.* (2005) AVP V1b selective antagonist SSR149415 blocks aggressive behaviors in hamsters. *Pharmacol. Biochem. Behav.* 80: 189-194.

3. Griebel G *et al.* (2002) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V_{1b} receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc. Natl. Acad. Sci. USA* 99: 6370-6375.

4. 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.