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PRODUCT DATASHEET

ChemiScreen™ $VPAC_1$ VIP/PACAP Family Receptor Membrane Preparation

CATALOG NUMBER:	HTS043M	**QUANTITY:**	200 units
LOT NUMBER:	JH1756024	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

Vasoactive intestinal peptide (VIP), a 28 amino acid peptide originally isolated by its vasodilation activity, binds to two class B GPCRs, $VPAC_1$ and $VPAC_2$, to exert its functions in the CNS, vasculature, immune system and adrenal medulla (Harmar et al., 1998). In the immune system, VIP is synthesized by mast cells and lymphocytes, and appears to inhibit inflammation and to shift the immune response toward a Th2 pathway (Delgado et al., 2004). In the heart, VIP is expressed by nerve fibers, where it modulates heart rate, and coronary blood flow (Henning and Sawmiller, 2001). $VPAC_1$ 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 $VPAC_1$ interactions with VIP. The membrane preparations exhibit a Kd of 1.2 nM for [^{125}I]-VIP. With 5 µg/well $VPAC_1$ Membrane Prep and 1.2 nM [^{125}I]-VIP, a greater than 7-fold signal-to-background ratio is obtained.

APPLICATIONS:

Radioligand binding assay

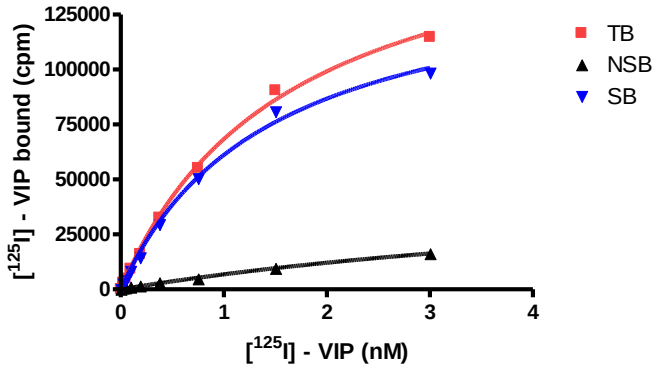

Figure 1. Saturation binding for $VPAC_1$. 5 µg/well $VPAC_1$ Membrane Preparation was incubated with increasing amount of ^{125}I-labeled VIP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled VIP. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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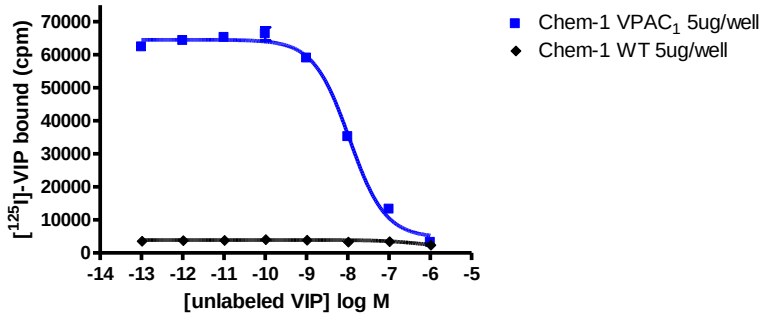

Figure 2. Competition binding for $VPAC_1$. 5 µg/well $VPAC_1$ Membrane Preparation and10 µg/well Wild-Type Chem-1 Membrane Preparation (EMD Millipore cat. # HTS000MC1) were incubated with 1.2 nM ^{125}I-labeled VIP and increasing concentrations of unlabeled VIP, and more than 7- fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 12.2 pmol/mg
K_d: 1.4 nM
Signal:background: ≥7-fold

TRANSFECTION: VIPR1 cDNA, encoding $VPAC_1$ (Accession number L13288)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous $VPAC_1$ 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: [^{125}I] VIP (Perkin Elmer# NEX192)

Wash Buffer: 50 mM Hepes, pH 7.4, 500 mM 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 7-fold signal:background with ^{125}I-labeled VIP at 1.2 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°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. Delgado M *et al*. (2004) The significance of vasoactive intestinal peptide in immunomodulation. Pharmacol. Rev. 56: 249-290.

2. Harmar AJ *et al*. (1998) International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. Pharmacol. Rev. 50: 265-270.
3. Henning RJ and Sawmiller DR (2001) Vasoactive intestinal peptide: cardiovascular effects. Cardiovasc. Res. 49: 27-37.

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