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PRODUCT DATASHEET

ChemiScreen™ $VPAC_2$ VIP/PACAP Membrane Preparation

CATALOG NUMBER:	HTS079M	**QUANTITY:**	200 units
LOT NUMBER:	SC191274	**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, $VPAC_2$ is expressed on stimulated CD4 T cells, and binding of T cell-derived VIP to $VPAC_2$ induces a shift toward the Th2 pathway (Delgado *et al*., 2004; Voice *et al*., 2004). In addition, $VPAC_2$ is an essential regulator of the circadian pacemaker of the hypothalamic suprachiasmatic nuclei (Hughes *et al*., 2004). $VPAC_2$ 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 $VPAC_2$ interactions with PACAP. The $VPAC_2$ receptor membrane preparations exhibit a Kd of 0.86 nM for [^{125}I]-PACAP27. With 0.5 nM [^{125}I]-PACAP27, 5 µg/well of $VPAC_2$ Receptor Membrane Prep yields greater than a 10-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

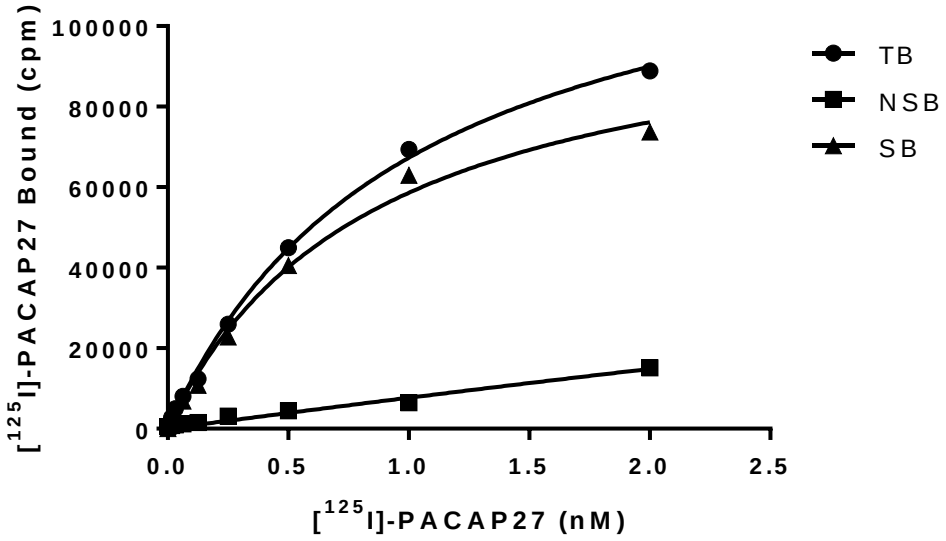

Figure 1. Saturation Binding for $VPAC_2$. 5 µg/well of $VPAC_2$ Membrane Preparation were incubated with increasing amounts of [^{125}I]-PACAP27 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled PACAP(1-27). Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC191274.

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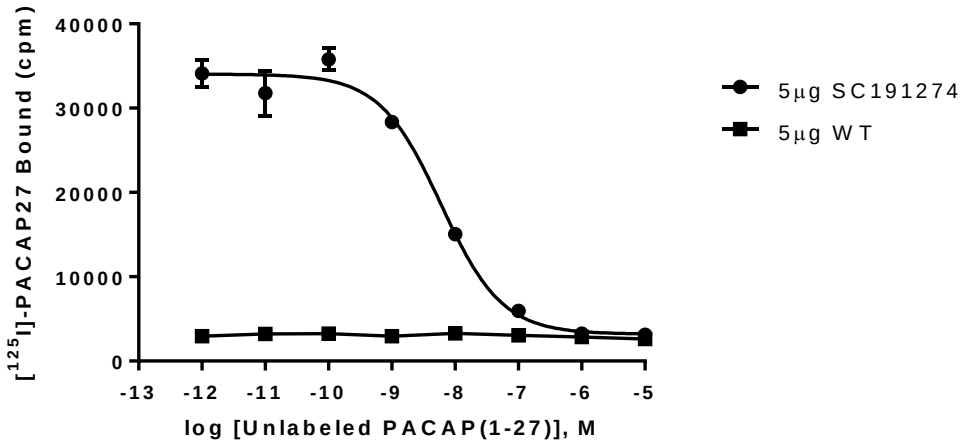

Figure 2. Competition Binding for $VPAC_2$. 5 µg/well of $VPAC_2$ Membrane Preparation or Wild-Type Chem-1 membrane preparation (Catalog # HTS000MC1) were incubated with 0.5 nM [^{125}I]-PACAP27 and increasing concentrations of unlabeled PACAP(1-27), and more than a 10-fold signal:background ratio was obtained. The data are from a representative sample of lot SC191274.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I]-PACAP27 Binding: 8.9 pmol/mg protein
K_d for [^{125}I]-PACAP27 Binding: 0.86 nM
Signal:Background: ≥10-fold

Species: Human $VPAC_2$ (Accession number NM_003382)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous $VPAC_2$ expression.

RECOMMENDED ASSAY CONDITIONS: Membranes were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate and incubated for 2 h at room temperature. Prior to filtration, an FC 96-well harvest plate was coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions were transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted for determination of receptor-associated radioligand binding.

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]-PACAP27 (PerkinElmer # NEX294)

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 a 10-fold signal:background ratio with [^{125}I]-PACAP27 at 0.5 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 1 mg/mL in packaging buffer, dispensed at 1 mL per vial, 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. 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. Hughes AT *et al.* (2004). Aberrant gating of photic input to the suprachiasmatic circadian pacemaker of mice lacking the $VPAC_2$ receptor. *J. Neurosci.* 24:3522-6.

4. Voice J *et al.* (2004). c-Maf and JunB mediation of Th2 differentiation induced by the type 2 G Protein-Coupled Receptor ($VPAC_2$) for vasoactive intestinal peptide. *J. Immunol.* 172:7289-7296.

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