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PRODUCT DATASHEET

ChemiScreen™ PAC_1-Long Membrane Preparation

CATALOG NUMBER:	HTS114M	**QUANTITY:**	200 units
LOT NUMBER:	2093604	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

PACAP (pituitary adenylyl cyclase-activating peptide) is a peptide that exists in 2 forms, 27 or 38 amino acids, and is related to vasoactive intestinal peptide (VIP). Three related class B GPCRs, PAC_1, $VPAC_1$ and $VPAC_2$, bind to PACAP; however, $VPAC_1$ and $VPAC_2$ have a much higher affinity for VIP than does PAC_1 (Vaudry et al., 2000). Several splice variants of PAC_1 result in proteins that differ at the N-terminus and third intracellular loop; these variants differ in their affinities for PACAP and abilities to activate Gq and Gs. High expression of PAC_1 is observed in the CNS and the adrenal medulla. Studies with PAC_1-null mice indicate that PAC_1 plays important roles in regulation of circadian rhythms, neutrophil migration, and pulmonary vascular tone (Hannibal et al., 2001; Martinez et al., 2005; Otto et al., 2004). PAC_1-long 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 PAC_1-long interactions with PACAP27. The membrane preparations exhibit a Kd of 2.7 nM for [^{125}I]-PACAP27. With 5 µg/well PAC1-long Membrane Prep and 0.75 nM [125I]-PACAP27, a greater than 12-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

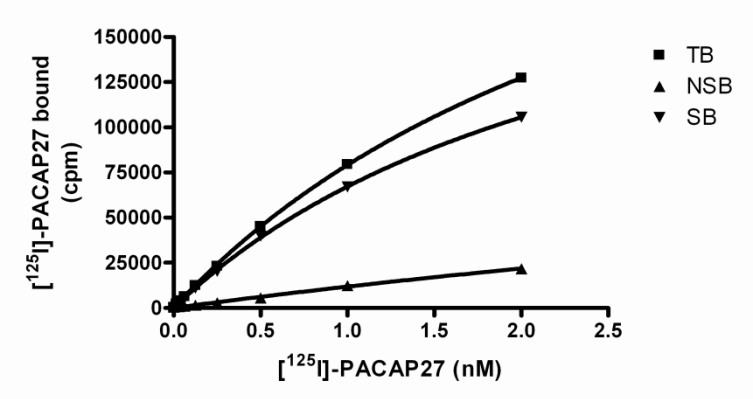

Figure 1. Saturation binding for PAC_1-long. 5 µg/well PAC_1-long Membrane Preparation was incubated with increasing amount of [^{125}I]-PACAP27 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled PACAP27. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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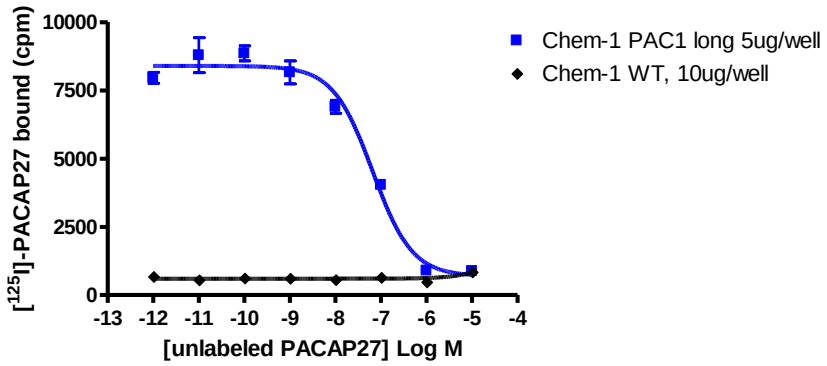

Figure 2. Competition binding for PAC_1-long. PAC_1-long Membrane Preparation (5 or 10 µg/well) or Wild-Type Chem-1 membrane preparation (Catalog # HTS000MC1) was incubated with 0.75 nM [125I]-PACAP27 and increasing concentrations of unlabeled PACAP27, and more than 12- fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 52.0 pmol/mg
K_d: 2.7nM
Signal:background: >12-fold

TRANSFECTION: Human ADCYAP1R1 cDNA encoding the long isoform of PAC_1 (Accession number NM_001118)

Species: Human

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

Radioligand: [125I] PACAP27 (Perkin Elmer # NEX294)

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 12-fold signal:background with 125I-labeled PACAP27 at 0.75 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) Hannibal J et al. (2001) Dissociation between light-induced phase shift of the circadian rhythm and clock gene expression in mice lacking the pituitary adenylate

cyclase activating polypeptide type I receptor. J. Neurosci. 21: 4883-4890.

2) Martinez C et al. (2005) Analysis of the role of the PAC1 receptor in neutrophil recruitment, acute-phase response, and nitric oxide production in septic shock. J. Leukoc. Biol. 77(5):729-38.

3) Otto C et al. (2004) Pulmonary hypertension and right heart failure in pituitary adenylate cyclase-activating polypeptide type I receptor-deficient mice. Circulation 110: 3245-3251.

4) Vaudry D et al. (2000) Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. Pharmacol. Rev. 52: 269-324.

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