



CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN PAC₁-LONG RECEPTOR

CATALOG NUMBER: HTS114M **QUANTITY:** 200 units
LOT NUMBER: **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, PAC₁, VPAC₁ and VPAC₂, bind to PACAP; however, VPAC₁ and VPAC₂ have a much higher affinity for VIP than does PAC₁ (Vaudry et al., 2000). Several splice variants of PAC₁ 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 G_q and G_s. High expression of PAC₁ is observed in the CNS and the adrenal medulla. Studies with PAC₁-null mice indicate that PAC₁ 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). Chemicon's PAC₁-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 PAC₁-long interactions with PACAP27. The membrane preparations exhibit a K_d of 2.7 nM for [¹²⁵I]-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

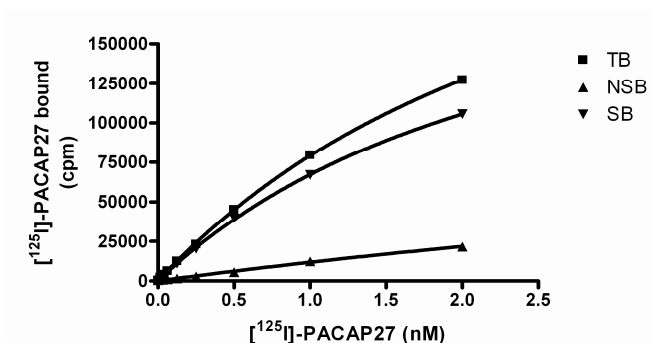


Figure 1. Saturation binding for PAC₁-long. 5 µg/well PAC₁-long Membrane Preparation was incubated with increasing amount of [¹²⁵I]-PACAP27 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled PACAP27. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

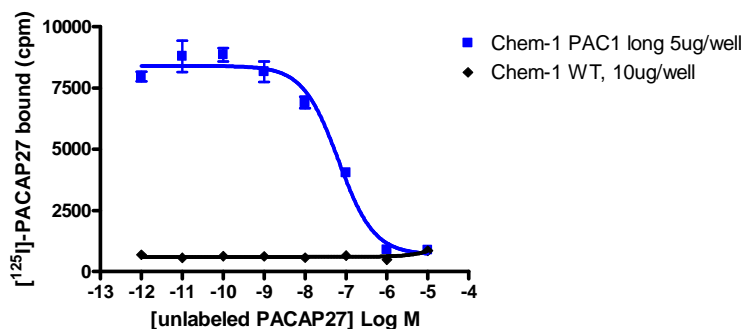


Figure 2. Competition binding for PAC₁-long. PAC₁-long Membrane Preparation (5 or 10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.75 nM [¹²⁵I]-PACAP27 and increasing concentrations of unlabeled PACAP27, and more than 12- fold signal:background was obtained. Representative sample data.

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

SPECIES: Human ADCYAP1R1 cDNA encoding the long isoform of PAC₁ (Accession number NM_001118)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous PAC₁ 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 an 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: Membranes protein were 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:

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.

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.

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.

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

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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