

# **Human PACT Antibody**

Antigen Affinity-purified Polyclonal Goat IgG Catalog Number: AF1980

DESCRIPTION		
Species Reactivity	Human	
Specificity	Detects human PACT in direct ELISAs and Western blots.	
Source	Polyclonal Goat IgG	
Purification	Antigen Affinity-purified	
Immunogen	E. coli-derived recombinant human PACT Ser2-Lys313 Accession # O75569	
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.	

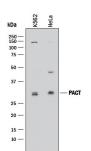
### APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

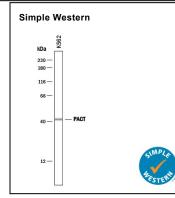
	Recommended Concentration	Sample
Western Blot	1 μg/mL	See Below
Simple Western	10 μg/mL	See Below

# Western Blot

DATA



Detection of Human PACT by Western Blot. Western blot shows lysates of K562 human chronic myelogenous leukemia cell line and HeLa human cervical epithelial carcinoma cell line. PVDF membrane was probed with 1 μg/mL of Goat Anti-Human PACT Antigen Affinity-purified Polyclonal Antibody (Catalog # AF 1980) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for PACT at approximately 32 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.



Detection of Human PACT by Simple Western<sup>TM</sup>. Simple Western lane view shows lysates of K562 human chronic myelogenous leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for PACT at approximately 42 kDa (as indicated) using 10 μg/mL of Goat Anti-Human PACT Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1980) followed by 1:50 dillution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

# PREPARATION AND STORAGE

Reconstitution Reconstitute at 0.2 mg/mL in sterile PBS

Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

\*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

# Stability & Storage

# Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

### BACKGROUND

PACT (PKR Activator; also HSD14, PRKRA and RAX [in mouse]) is a 34-37 kDa member of the PRKRA family of molecules. It is a ubiquitously expressed, cytoplasmic, 313 amino acid (aa) protein that contains three discrete double-stranded (ds) RNA binding motifs (aa 31-101; 126-194; 240-308). It exists as a monomer, homodimer, and heterodimer with at least two partners, TRBP and Dicer. Two isoform variants are known to exist, including one that utilizes an alternative start site at Met26, and another that shows a 10 aa substitution for aa 1-21. PACT is perhaps best known for its relationship with PKR, a 52-58 kDa Ser/Thr kinase that downmodulates protein synthesis and initiates apoptosis. PKR is constitutively silent, and activated via two principal ways; the first is through binding to (viral) dsRNA, and the second is through binding to an activator, PACT. PACT is kept in check upstream through dimerization with at 48-49 kDa regulatory protein termed TRBP. Following growth factor withdrawl or exposure to bacterial products such as LPS, PACT is phosphorylated at Ser287, and this inactive PACT:TRBP heterodimer dissociates, freeing up PACT to bind to PKR. PACT's interaction with PKR induces an autophosphorylation event on PKR. This allows for an activated PKR interaction with elF2a, causing its phosphorylation and a subsequent reduction of protein synthesis. PACT may also act independently of PKR, and serve as a component of RISC, a complex that downregulates mRNA translation. Within this complex, PACT likely associates with Dicer in concert with Argonaute. Full-length PACT shares 98% aa sequence identity with the mouse ortholog to human PACT termed RAX.

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