

Certificate of Analysis

Product: Affinity Purified Anti-Pdcd4 pS457 [Rabbit]

Code: 600-401-964

Lot # 19707

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 0.95 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background Information: Programmed cell death 4 (Pdcd4) is a novel tumor suppressor. Pdcd4 directly inhibits the helicase activity of eukaryotic translation initiation factor 4A (eIF4A), a component of the translation initiation complex. Pdcd4 also suppresses the transactivation of activator protein-1 (AP-1)-responsive promoters by c-Jun. Pdcd4 contains two Akt phosphorylation sites, one at Ser67 and the other at Ser457. The phosphorylation of Pdcd4 by Akt causes nuclear translocation of Pdcd4 and a significant decrease in the ability of Pdcd4 to interfere with the transactivation of AP-1-responsive promoters by c-Jun.

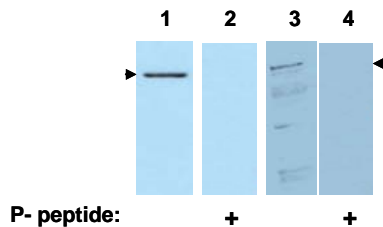


Figure 1. Western blot using Rockland's affinity purified anti-Pdcd4 pS457 antibody shows detection of Pdcd4 phosphorylated at Ser 457 (arrowheads). Lanes 1 & 2 each contain 100 ng recombinant Pdcd4. Lanes 3 & 4 each contain 30 µg of whole cell extract from 293 HEK cells treated with 20 nM TPA and MG132 proteasome inhibitor for 8 hours. The signal can be competed off with peptide phosphorylated at Ser 457 (Lanes 2 & 4). *Personal Communication, M Young & A Jansen, NCI, Bethesda, MD.*

Application Note(s): This affinity purified antibody has been tested for use in ELISA and western blotting. Specific conditions for reactivity should be optimized by the end user. By western blot, a band approximately 52 kDa in size corresponding to Pdcd4 protein is expected in the appropriate cell lysate or extract.

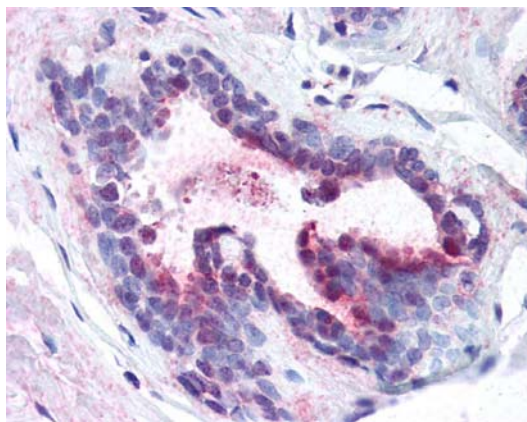


Figure 2. Immunohistochemistry. Rockland's affinity purified anti-Pdcd4 pS457 antibody was used at 1.25 µg/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate positive staining of human breast epithelial cells at 40X. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. *Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.*

Recommended Dilutions:	ELISA	1:400,000
	WESTERN BLOT	1:500 - 1:2,000
	IMMUNOHISTOCHEMISTRY	21.25 - 2.5 µg/ml
	OTHER APPLICATIONS	User Optimized

Purity and Specificity: This product was affinity purified from monospecific antiserum by immunoaffinity chromatography using phospho-peptide coupled to agarose beads followed by solid phase adsorption against nonphospho-peptide. This antibody is specific for human Pdcd4 protein phosphorylated at Ser457. A BLAST analysis was used to suggest cross-reactivity with Pdcd4 from human, mouse, rat and *Xenopus* based on 100% homology with the immunizing sequence. Cross-reactivity with Pdcd4 from other sources has not been determined.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids surrounding Ser457 in the human Pdcd4 protein.

Relevant Links: NCBI [NP_055271](#) Swiss-Prot [O15501](#)

General References:

Jansen AP, Camalier CE, Colburn NH (2005) Epidermal expression of the translation inhibitor programmed cell death 4 suppresses tumorigenesis. *Cancer Res.* **65**(14):6034-6041.

Palamarchuk A, Efanov A, Maximov V, Aqeilan RI, Croce CM, Pekarsky Y (2005) Akt phosphorylates and regulates Pdcd4 tumor suppressor protein. *Cancer Res.* **65** (24):11282-11286.

Related Products:

#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122	IRDye800™ Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#611-144-122	DyLight™680 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#B501-0500	BLOTTO (500 g)
#BSA-30	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
#MB-070	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	MaxTag ™ Anti-RABBIT IgG Kit for Immunoblotting

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.