

## Datasheet for 600-401-964

**Pdcd4 phospho S457 Antibody****Overview**

<b>Description:</b>	Anti-Pdcd4 pS457 (RABBIT) Antibody - 600-401-964
<b>Item No.:</b>	600-401-964
<b>Size:</b>	100 µg
<b>Applications:</b>	ELISA, IHC, WB
<b>Reactivity:</b>	Human
<b>Host Species:</b>	Rabbit

**Product Details**

<b>Background:</b>	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. 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.
<b>Synonyms:</b>	rabbit anti-PDCD4 pS457 antibody, PDCD-4, PDCD 4, Programmed cell death protein 4, Death up-regulated gene protein antibody, Dug antibody, H731 antibody, Ma3 antibody, Neoplastic transformation inhibitor antibody, Neoplastic transformation inhibitor protein antibody, Nuclear antigen H731 antibody, Protein 197/15a
<b>Host Species:</b>	Rabbit
<b>Clonality:</b>	Polyclonal
<b>Format:</b>	IgG

**Target Details**

<b>Gene Name:</b>	PDCD4
<b>Reactivity:</b>	Human

<b>PTM Specificity:</b>	Phosphorylation
<b>Immunogen Type:</b>	Conjugated Peptide
<b>Immunogen:</b>	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.
<b>Purity/Specificity:</b>	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.
<b>Relevant Links:</b>	<ul style="list-style-type: none"><li>• <a href="#">UniProtKB - Q53EL6</a></li><li>• <a href="#">NCBI - 21735596</a></li><li>• <a href="#">GeneID - 27250</a></li></ul>

## Application Details

<b>Tested Applications:</b>	ELISA, IHC, WB
<b>Application Note:</b>	This affinity purified antibody has been tested for use in ELISA, immunohistochemistry, 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.
<b>Assay Dilutions:</b>	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
<b>ELISA:</b>	1:400,000
<b>IHC:</b>	21.25 - 2.5 µg/ml
<b>WB:</b>	1:500 - 1:2,000

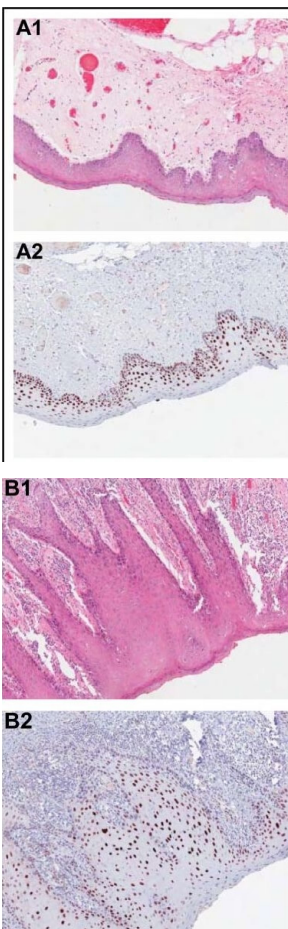
## Formulation

<b>Physical State:</b>	Liquid (sterile filtered)
<b>Concentration:</b>	1.0 mg/mL by UV absorbance at 280 nm
<b>Buffer:</b>	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
<b>Preservative:</b>	0.01% (w/v) Sodium Azide
<b>Stabilizer:</b>	None

## Shipping & Handling

<b>Shipping Condition:</b>	Dry Ice
<b>Storage Condition:</b>	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
<b>Expiration:</b>	Expiration date is one (1) year from date of receipt.

## Images

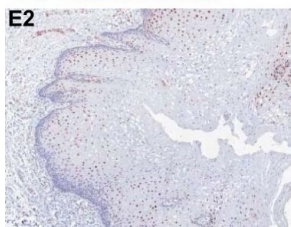
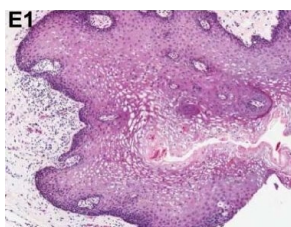
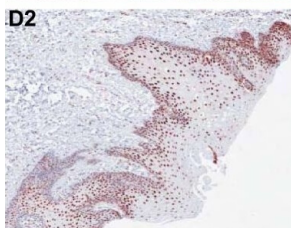
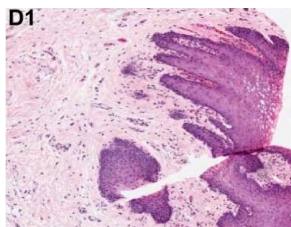
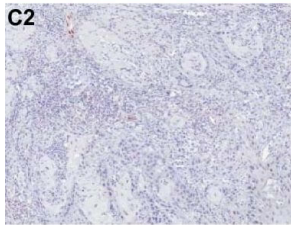
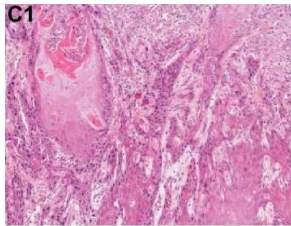


### Immunohistochemistry

Immunohistochemical analysis of PDCD4 shows the corresponding H&E-stained and PDCD4-stained tissue sections from patients with OSCC. Panels A1, A2, D1, D2 show two adjacent normal epithelium samples with strongly positive, nuclear PDCD4 staining. Panels B1, B2, E1, E2 show two dysplasia samples with positive to weak nuclear PDCD4 staining. Panels C1, C2, F1, F2 show loss of PDCD4 expression in two moderately differentiated OSCCs. Normal, dysplasia and OSCC samples are paired and correspond to two different patients (A-C and D-F, respectively). Figure provided by CiteAb. Source: Mol Cancer, PMID: 20831814.

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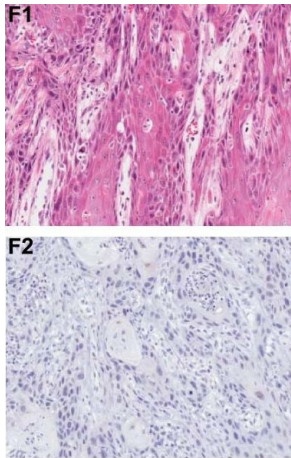
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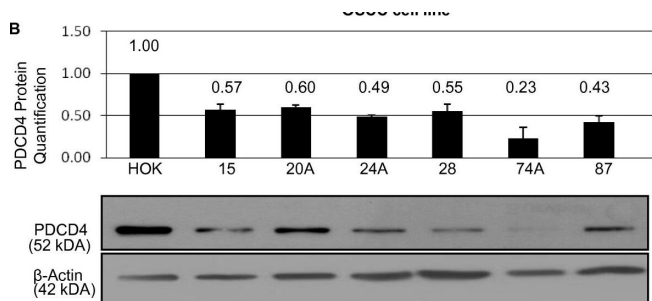
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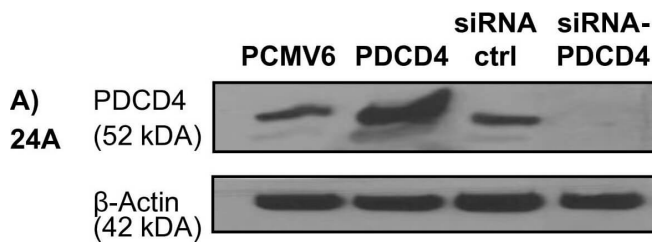
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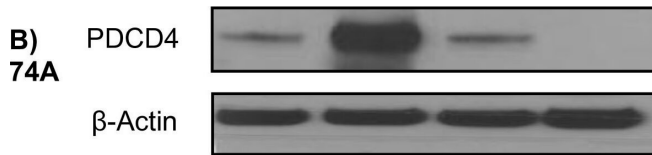
### Western Blot

PDCD4 mRNA and PDCD4 protein levels in OSCC cell lines. (A) The log<sub>10</sub> ratio of PDCD4 mRNA in OSCC cell lines relative to HOK. (B) Quantification of PDCD4 protein expression in OSCC cell lines with a representative Western blot of PDCD4 protein in OSCC cell lines below. Cell line data are plotted mean ± SE and are representative of 3 separate experiments. Figure provided by CiteAb. Source: Mol Cancer, PMID: 20831814.



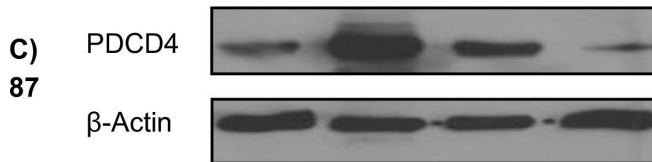
### Western Blot

Western blotting analysis demonstrating over-expression or knock-down of PDCD4 using PDCD4 plasmid or PDCD4 targeted siRNA, respectively, versus control plasmids (PCMV6, siRNA ctrl) in the UT-SCC cell lines (A) 24A, (B) 74A, and (C) 87. Figure provided by CiteAb. Source: Mol Cancer, PMID: 20831814.



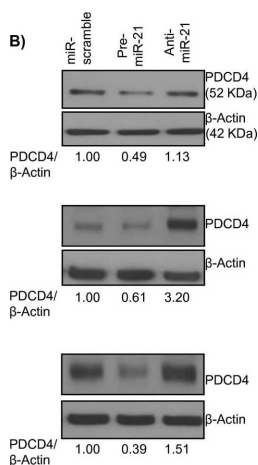
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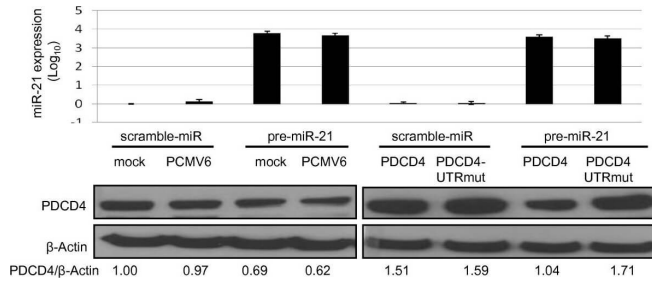
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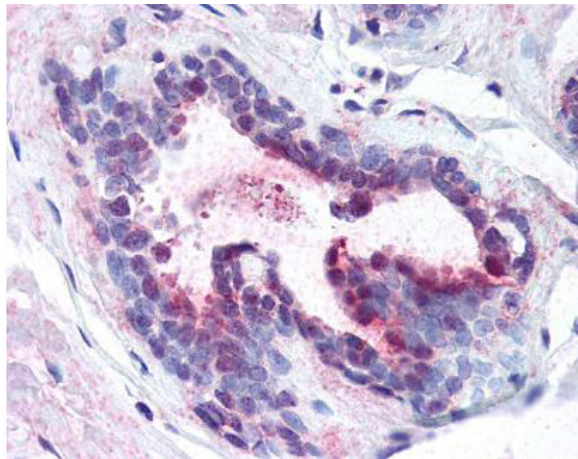
**Western Blot**

Panel A shows miR-21 expression in pre-miR-21 or anti-miR-21 transfected cells compared to control (miR-scramble) in the UT-SCC cell lines 24A, 74A, and 87. Panel B shows PDCD4 protein levels (Western blot) after transfection with pre-miR-21 or anti-miR-21 compared to miR-scramble control. PCR data plotted are the mean  $\pm$  SE and are representative of 3 separate experiments. In the Western blot, PDCD4/ $\beta$ -Actin represents the ratio of the band intensity of PDCD4 compared to that of  $\beta$ -Actin, and are shown below the blots, for each cell line. Panels A-C in the same line corresponds to the same cell line, in this order (UT-SCC-24A, 74A and 87). Figure provided by CiteAb. Source: Mol Cancer, PMID: 20831814.



### Western Blot

The upper panel shows miR-21 expression levels following transfection with pre-miR21, PDCD4 and PDCD4-UTRmut, compared to controls: scramble miR and PCMV6 empty vector. miR-21 expression data are presented as Log<sub>10</sub> fold change, compared to mock-transfected control. Data are plotted as mean ± SE and are representative of two separate experiments. The lower panel shows the Western blot analysis of PDCD4 protein levels for the different transfection conditions. PDCD4/β-Actin represents the ratio of the band intensity of PDCD4 compared to that of β-Actin, and is shown below each blot. Co-transfection of miR-21 with PDCD4, but not PDCD4-UTRmut, resulted in a decrease in PDCD4 protein expression. Figure provided by CiteAb. Source: Mol Cancer, PMID: 20831814.

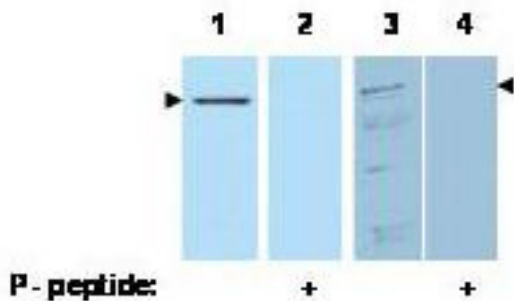


### Immunohistochemistry

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.

### Western Blot

Western blot using Rockland's affinity purified anti-Pdcd4 pS457 antibody shows detection of Pdcd4 phosphorylated at Ser 457 (lanes 1 & 3) at ~52kDa (arrow). 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.



## References

- Reis PP et al. Programmed cell death 4 loss increases tumor cell invasion and is regulated by miR-21 in oral squamous cell carcinoma. *Mol Cancer*. (2010)

## Disclaimer

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 5199, Limerick, Pennsylvania, USA.