

## Product Datasheet

### PDCD4 Antibody (orb345560)

<b>Catalog Number</b>	orb345560
<b>Category</b>	Antibodies
<b>Description</b>	Pdcd4 (phospho-S457) antibody
<b>Target</b>	PDCD4
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human
<b>Form/Appearance</b>	Liquid (sterile filtered)
<b>Concentration</b>	1.0 mg/mL
<b>Buffer/Preservatives</b>	Preservative: 0.01% (w/v) Sodium Azide. Stabilizer: None; Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
<b>Purity</b>	Anti-Pdcd4 pS457 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.

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<b>Immunogen</b>	Anti-Pdcd4 pS457 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>UniProt ID</b>	<b>Q53EL6</b>
<b>Tested applications</b>	ELISA, IHC, KO/KD Validated, WB
<b>Dilution range</b>	ELISA: 1:400,000, IHC: 1.25 - 2.5 µg/ml, WB: 1:500 - 1:2,000
<b>Application notes</b>	Anti-Pdcd4 pS457 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>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
<b>Dry Ice Shipping</b>	<b>Please note: This product requires shipment on dry ice. A dry ice surcharge will apply.</b>
<b>Note</b>	For research use only
<b>NCBI</b>	<b>21735596</b>
<b>Expiration Date</b>	12 months from date of receipt.

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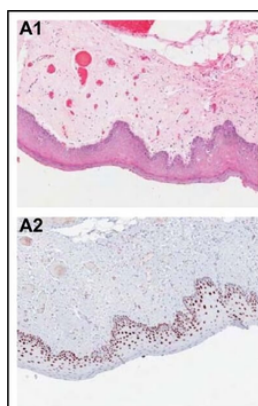
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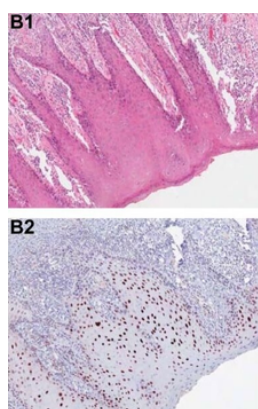
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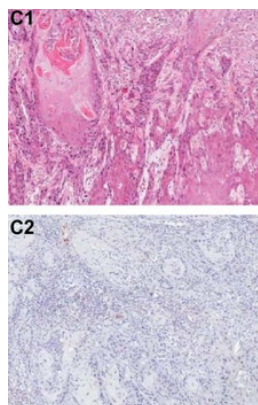
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Immunohistochemical analysis of PDCD4 shows the corresponding H&E-stained and PDCD4-stained tissue sections from patients with OSCC. Panels A1, A2, D1, D2 show two adjacent normal epithelium samples with strongly positive, nuclear PDCD4 staining. Panels 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).



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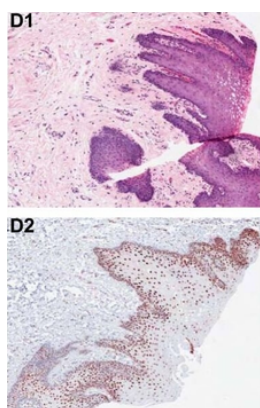
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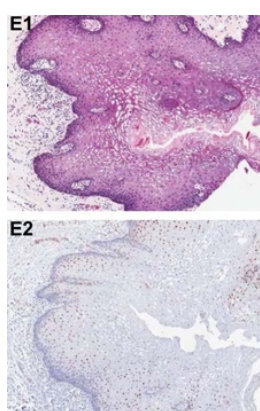
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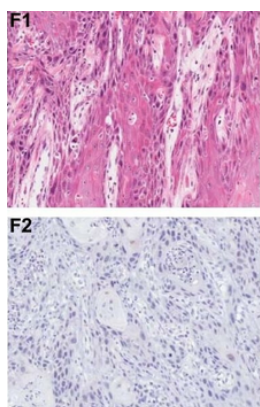
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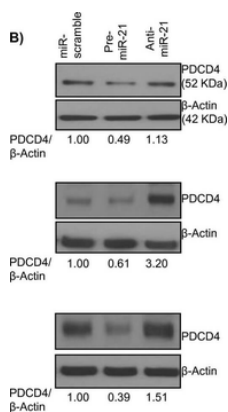
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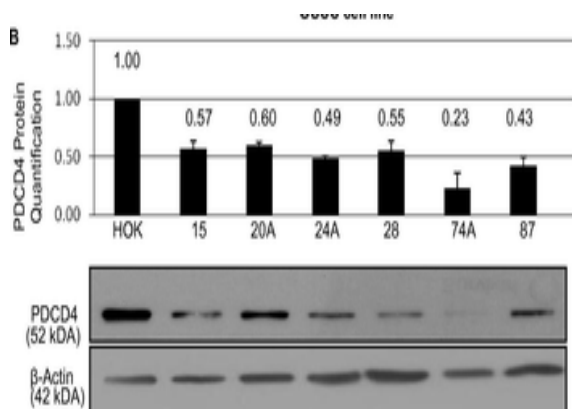
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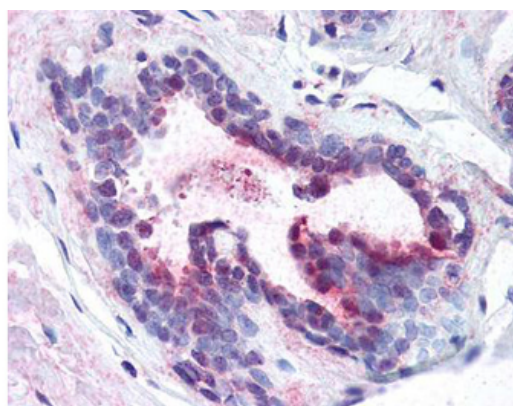
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Panel A shows miR-21 expression in pre-miR-21 or anti-miR-21 transfected cells compared to control (miR-scramble) in the UT-SCC cell lines 24A, 74A, and 87. Panel B shows PDCD4 protein levels (Western blot) after transfection with pre-miR-21 or anti-miR-21 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).



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  $\pm$  SE and are representative of 3 separate experiments.



Biorbyt's affinity purified anti-Pdcd4 pS457 antibody was used at 1.25  $\mu$ g/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.

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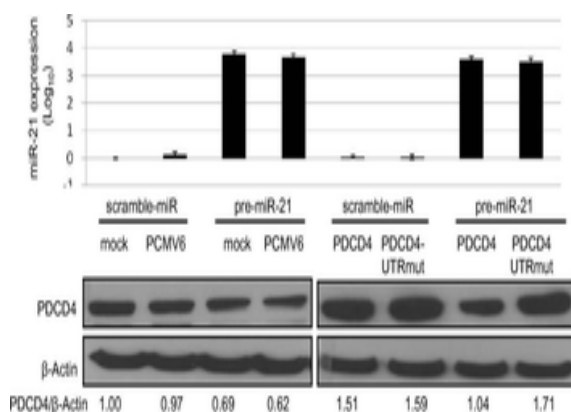
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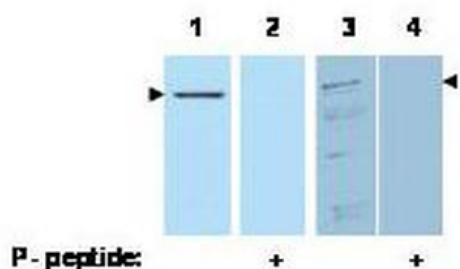
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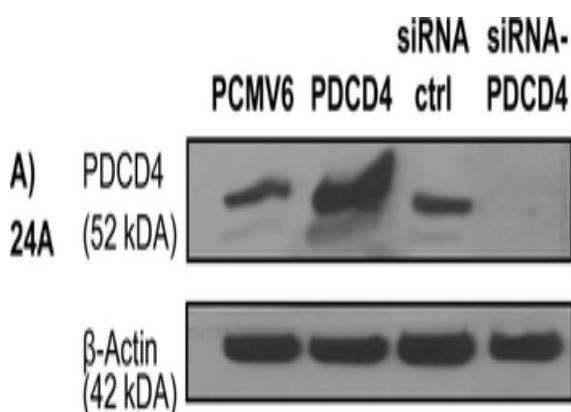
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The upper panel shows miR-21 expression levels following transfection with pre-miR21, PDCD4 and PDCD4-UTRmut, compared to controls: scramble miR and PCMV6 empty vector. miR-21 expression data are presented as Log<sub>10</sub> fold change, compared 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.



Western blot using Biorbyt's affinity purified anti-Pdcd4 pS457 antibody shows detection of Pdc4 phosphorylated at Ser 457 (lanes 1 & 3) at ~52kDa (arrow). Lanes 1 & 2 each contain 100 ng recombinant Pdc4. 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).



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.

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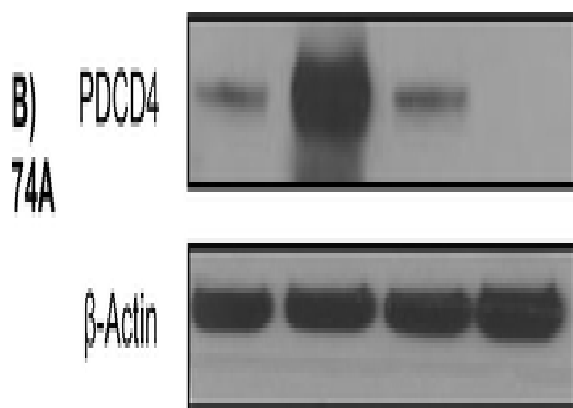
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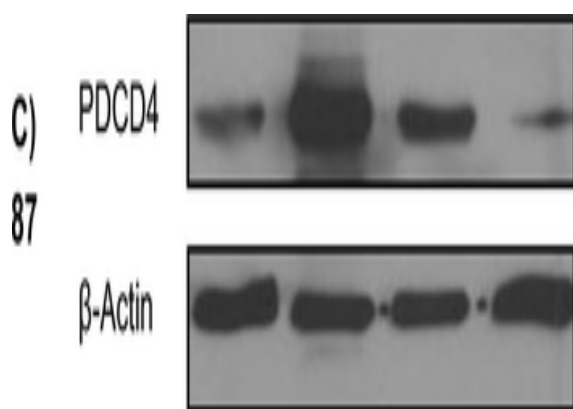
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