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Datasheet for 600-401-965

Pdcd4 Antibody

Overview

Description:	Anti-Pdcd4 (RABBIT) Antibody - 600-401-965
Item No.:	600-401-965
Size:	100 µg
Applications:	ELISA, IHC, WB, IP, Microarray, Other
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	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.
Synonyms:	rabbit anti-PDCD4 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
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	PDCD4
Reactivity:	Human, Mouse

Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding amino acids near the carboxyl terminus of human Pdcd4 protein.
Purity/Specificity:	This affinity purified antibody is directed against human Pdcd4 protein. The product was affinity purified from monospecific antiserum by immunoaffinity chromatography. 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. The antibody reacts with Pdcd4 protein that is either phosphorylated or non-phosphorylated at Ser457.
Relevant Links:	• UniProtKB - Q53EL6 • NCBI - 21735596 • GeneID - 27250

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IP, Microarray, Other (Based on references)
Application Note:	This affinity purified antibody has been tested for use in ELISA, western blotting, immunoprecipitation and immunohistochemistry. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 52 kDa in size corresponding to Pdcd4 protein by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:100,000
IHC:	1:100 – 1:300
WB:	1:1,000 - 1:10,000

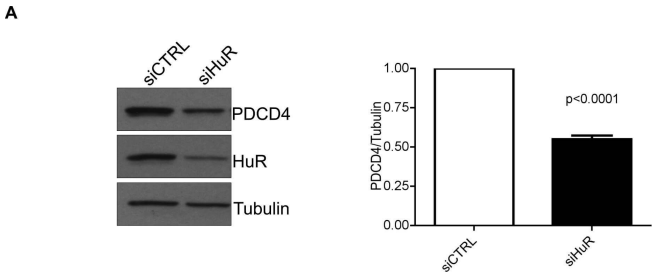
Formulation

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

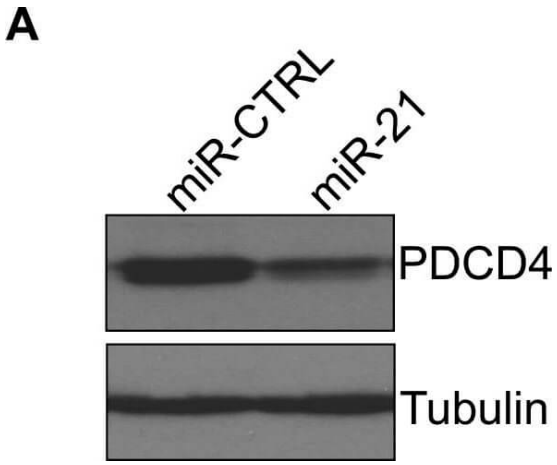
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	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.
Expiration:	Expiration date is one (1) year from date of receipt.

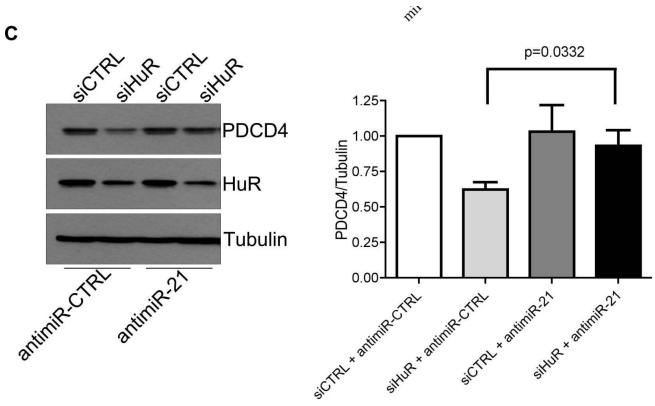
Images



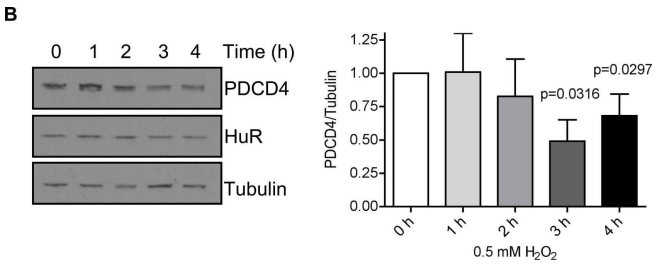
Western Blot
HuR directly binds to PDCD4 3'UTR mRNA to regulate its protein expressionA. Left panel: Western blot analysis of PDCD4 protein levels after HuR knockdown. HeLa cells were treated with siHuR or siCTRL (non-targeting control) for 72 h and harvested for western blot analysis. Tubulin was used as a loading control. Right panel: PDCD4 protein levels are quantified relative to Tubulin. B. HeLa cells were treated with siHuR or siCTRL for 72 h, harvested, and total RNA was isolated. PDCD4 mRNA levels were quantified by qRT-PCR and are shown relative to GAPDH mRNA levels. C. Seventy-two hours after siRNA transfection, HeLa cells were treated with 5 µg/mL actinomycin D. After the chase period, cells were processed for qRT-PCR to determine the mRNA half-life (11.6h for siCTRL; 9.5h for siHuR). D. Top panel: HeLa cells were crosslinked with formaldehyde and endogenous HuR was immunoprecipitated with mouse anti-HuR antibody; IgG was used as a control. Western blot analysis shows the level of immunoprecipitated HuR. Bottom panel: HuR-bound RNA was isolated and quantified by qRT-PCR, and is shown relative to IgG-immunoprecipitated material. The levels of GAPDH and RPL13 in HuR immunoprecipitation were determined as specificity controls E. PDCD4 3'UTR RNA was in vitro transcribed, 32P labelled and UV crosslinking was performed with recombinant GST (control) or GST-HuR, separated by SDS-PAGE, and exposed to X-Ray film. Figure provided by CiteAb. Source: Oncotarget, PMID: 26595526.



Western Blot
HuR regulates PDCD4 stability via miR-21A. HeLa cells were transiently transfected with a miR-21 mimic for 24 h and cells were harvested for western blot analysis. Tubulin was used as a loading control. B. HeLa cells were transiently transfected with a miR-21 mimic for 24 h and RNA was harvested. qRT-PCR analysis showing decrease of PDCD4 mRNA relative to GAPDH after miR-21 over-expression. C. Left panel: AntimiR-21 or antimiR-CTRL (control) was transiently transfected into HeLa cells for 24 h followed by siHuR transfection for an additional 48 h. Cells were harvested and protein levels were analyzed by western blot. Right panel: Quantification of PDCD4 protein levels relative to Tubulin. Figure provided by CiteAb. Source: Oncotarget, PMID: 26595526.

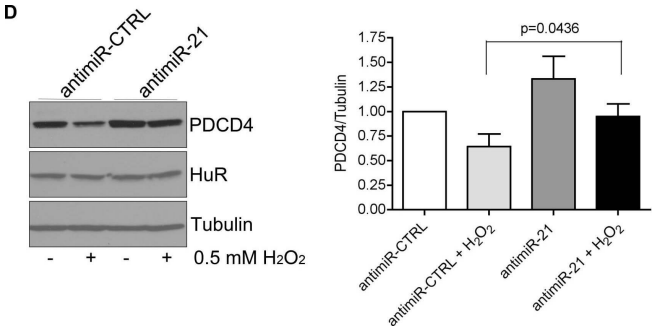


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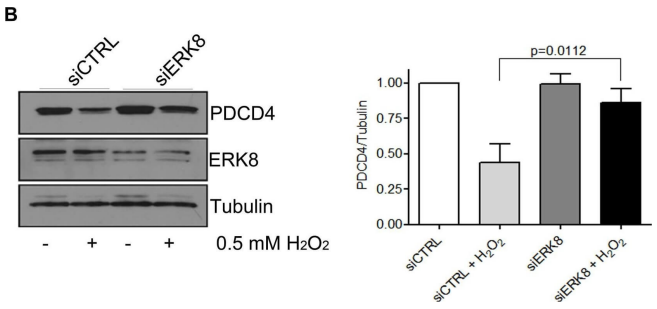
Western Blot

H2O2 causes cytoplasmic accumulation of HuR and a loss in PDCD4 expression that is mediated by miR-21A. HuR localization by immunofluorescence of HeLa cells treated with PBS (0 mM H2O2) or 0.5 mM H2O2 for 1 h. Nuclei are visualized by Hoechst staining. Nuclear/Cytoplasmic ratio of HuR is shown on the right. Higher ratio denotes more nuclear staining. B. Left panel: HeLa cells were treated with 0.5 mM H2O2 for the indicated times and cell lysates analysed by western blot analysis indicating a decrease in PDCD4 protein at 3 h as compared to Tubulin control. Right panel: PDCD4 protein levels were quantified relative to Tubulin. C. Cells were treated with 0.5 mM H2O2 for the indicated time points, total RNA was isolated and analysed by qRT-PCR indicating a loss of PDCD4 mRNA as compared to GAPDH control. D. Left panel: HeLa cells were treated with antimiR-21 or a non-targeting antimiR-CTRL (control) for 24 h followed by treatment with 0.5 mM H2O2 for 4 h. Cells were harvested and analysed by western blot analysis. Tubulin was used as a loading control. Right panel: Quantification of PDCD4 levels relative to Tubulin. E. HeLa cells were treated with 0.5 mM H2O2 or PBS and HuR was immunoprecipitated. Bound RNA was isolated and qRT-PCR was performed to determine levels of PDCD4 mRNA. The levels of HuR-bound PDCD4 in PBS-treated cells were set as 1. Figure provided by CiteAb. Source: Oncotarget, PMID: 26595526.



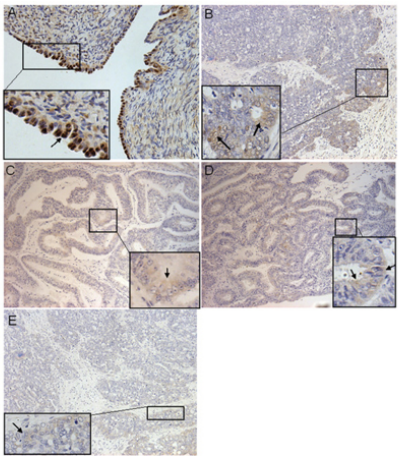
Western Blot

H2O2 causes cytoplasmic accumulation of HuR and a loss in PDCD4 expression that is mediated by miR-21A. HuR localization by immunofluorescence of HeLa cells treated with PBS (0 mM H2O2) or 0.5 mM H2O2 for 1 h. Nuclei are visualized by Hoechst staining. Nuclear/Cytoplasmic ratio of HuR is shown on the right. Higher ratio denotes more nuclear staining. B. Left panel: HeLa cells were treated with 0.5 mM H2O2 for the indicated times and cell lysates analysed by western blot analysis indicating a decrease in PDCD4 protein at 3 h as compared to Tubulin control. Right panel: PDCD4 protein levels were quantified relative to Tubulin. C. Cells were treated with 0.5 mM H2O2 for the indicated time points, total RNA was isolated and analysed by qRT-PCR indicating a loss of PDCD4 mRNA as compared to GAPDH control. D. Left panel: HeLa cells were treated with antimiR-21 or a non-targeting antimiR-CTRL (control) for 24 h followed by treatment with 0.5 mM H2O2 for 4 h. Cells were harvested and analysed by western blot analysis. Tubulin was used as a loading control. Right panel: Quantification of PDCD4 levels relative to Tubulin. E. HeLa cells were treated with 0.5 mM H2O2 or PBS and HuR was immunoprecipitated. Bound RNA was isolated and qRT-PCR was performed to determine levels of PDCD4 mRNA. The levels of HuR-bound PDCD4 in PBS-treated cells were set as 1. Figure provided by CiteAb. Source: Oncotarget, PMID: 26595526.



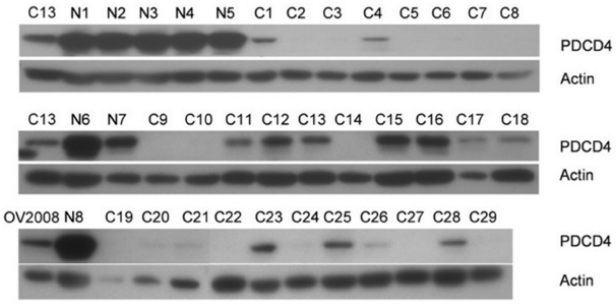
Western Blot

ERK8 phosphorylates HuR to prevent its binding to PDCD4 mRNAA. ERK8 or control siRNA was transfected into HeLa cells for 48 h followed by treatment of cells with 0.5 mM H2O2 or PBS for 1 h. Cells were fixed and immunofluorescence was performed to monitor HuR localization. Hoechst was used to stain the nuclei. Nuclear/Cytoplasmic ratio of HuR is shown on the right. Higher ratio denotes more nuclear staining. B. Top panel: HeLa cells were treated as in (A) and cells were harvested for western blot analysis for indicated proteins. Bottom panel: Quantification of PDCD4 protein levels relative to Tubulin. C. The kinase assay was performed with immunoprecipitated Flag-HuR or Flag empty vector as substrate and HA-ERK8 kinase in the presence of 32P gamma-ATP and exposed to X-ray film. The levels of HuR and ERK8 proteins were detected by western blot analysis. Figure provided by CiteAb. Source: Oncotarget, PMID: 26595526.

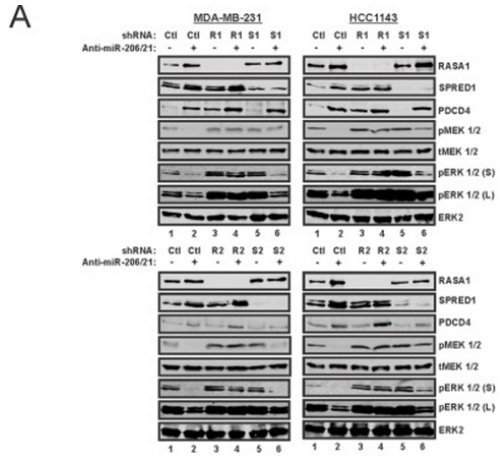


Immunohistochemistry

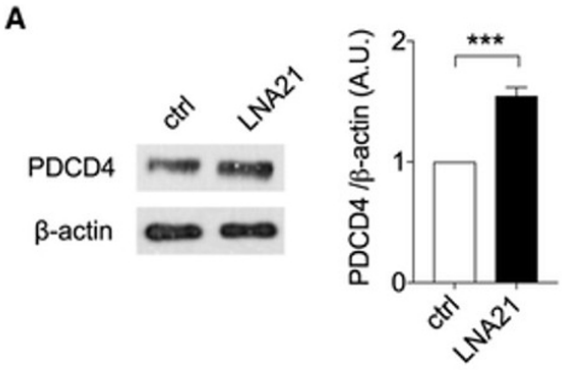
Representative examples of immunohistochemical staining of Pdcd4 in ovarian tissues. (A) Distinct nuclear staining was found in normal ovarian surface epithelium while much weaker cytoplasmic staining was found in (B) serous (C) mucinous, (D) endometrioid and (E) poorly differentiated adenocarcinomas. Arrows indicated representative positive staining sites. Fig 1. PMID: 19728867



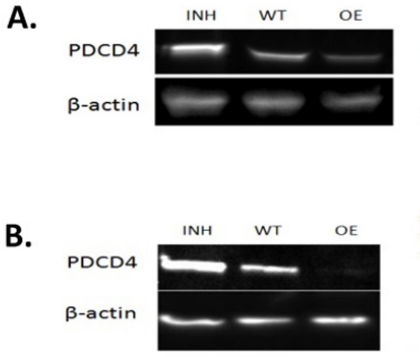
Western Blot
Western blot analysis of Pdcd4 expression in normal ovarian and malignant ovarian tissue samples. A representative of 8 normal samples (N1-8) and 29 malignant samples (C1-29) indicated significant higher Pdcd4 protein expression in normal compared with malignant ovarian tissue samples. Ovarian cancer cell line C13 or OV2008 were included as positive control and β-actin as loading control. Fig 3.
PMID: 19728867



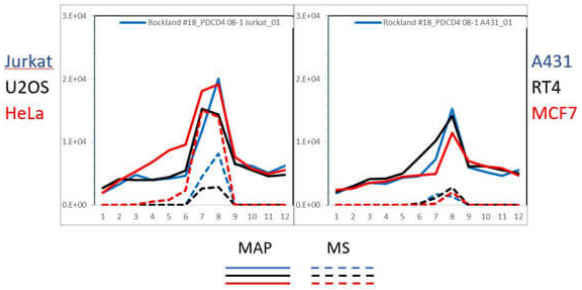
Western Blot
RASA1 and SPRED1 mediate the regulation of RAS-ERK pathway signaling by miR-206 and miR-21. (A) TNBC cells expressing the indicated shRNAs were treated with anti-miR-206 and anti-miR-21 in combination (anti-miR-206/21) or with anti-miR-Ctl. Whole-cell extracts were prepared, and the indicated proteins were analyzed by immunoblotting. Two distinct cell culture models were analyzed (MDA-MB-231 versus HCC1143) using independent shRNAs (R1, S1, R2, and S2). For pERK 1/2, both short (S) and long (L) exposures are indicated. Fig 11.
PMID: 25202123



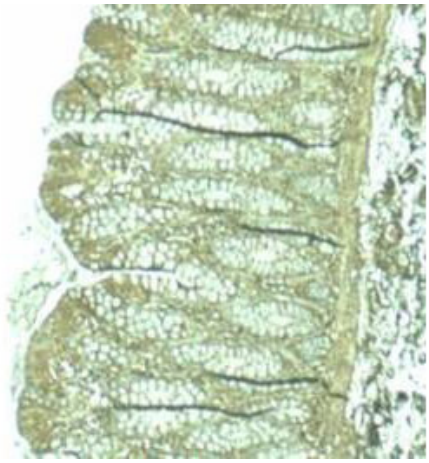
Western Blot
(A) Naive CD4+ T cells were transfected with either scrambled control or miR-21-blocking locked nucleic acid (LNA21). After 48 hr, PDCD4 and b-actin expression were assessed by western blot. Representative blots and mean normalized intensities from four experiments are shown (mean ± SEM, two-tailed paired t test). Fig 4.
PMID: 30463012



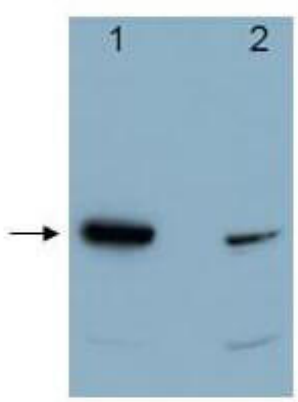
Western Blot
Expression of PDCD4 mRNA and protein in Rb1 and Y79 cells when transfected by miR-21 mimic or inhibiotor. (A) Rb1 and (B) Y79 cells were transfected with miR-21 mimic and inhibitor respectively. Two days after transfection, total RNA and protein were isolated. qRT-PCR showed the inhibition and overexpression were successful in comparison with the wild type control. PDCD4 mRNA levels in Rb1 cells did not show a significant change, but a significant fold change was present in Y79 cell data. Western blotting revealed PDCD4 proteins in both cells as having an inverse correlation with the miR-21 level, in which miR-21 overexpression reduced the PDCD4 protein while miR-21 inhibition restored the PDCD4 protein level. Fig 5. PMID: 25520758



PAGE-MAP
PAGE-MAP (microsphere affinity proteomics) of Rabbit Anti-PDCD4 Antibody. (Catalog Number: 600-401-965, Lot Number: 33416). Antibody array western blot binding of gelfree size separated fractions of multiple lysates (solid lines) and shotgun mass spectroscopy identification (dashed lines) of the target band run in parallel correlate confirming the specificity of this antibody against PDCD4. Data was provided by the Lund-Johansen lab of Oslo University Hospital. For more information on PAGE-MAP/IP-MS identification of antibody specificity and its large-scale implementation for antibody validation see Sikorski et. al., (2018) Nature Methods 15, 909-912.

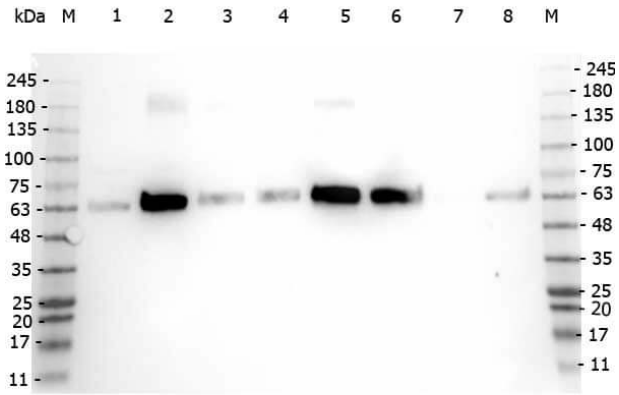


Immunohistochemistry
Affinity purified anti-Pdcd4 was used at a 1:100 dilution to detect Pdcd4 by immunohistochemistry on mouse colon tissue. Tissue was fixed in 4% paraformaldehyde and paraffin embedded. Tissue sections were deparaffinized and treated by trypsinization before staining. Personal Communication. M Young, NCI, Bethesda, MD.



Western Blot

Western blot using Rockland's affinity purified anti-Pdcd4 antibody shows detection of a band ~52 kDa in size corresponding to Pdcd4 (arrowhead). Lane 1 contains recombinant Pdcd4. Lane 2 contains 293 HEK cells treated with TPA and MG132. The anti-Pdcd4 antibody was used at a 1: 5,000 dilution. Personal Communication. M Young & A Jansen, NCI, Bethesda, MD.



Western Blot

Western Blot of Rabbit anti-PDCD antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: HEK293 lysate (p/n W09-000-365). Lane 2: HeLa Lysate (p/n W09-000-363). Lane 3: MCF-7 Lysate (p/n W09-000-360). Lane 4: Jurkat Lysate (p/n W09-000-370). Lane 5: A431 Lysate (p/n W09-000-361). Lane 6: Raji Lysate (p/n W09-001-368). Lane 7: Ramos Lysate (p/n W09-000-GK4). Lane 8: NIH/3T3 Lysate (p/n W10-000-358). Load: 35 µg per lane. Primary antibody: PDCD antibody at 1:1,000 for 3hrs at RT. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS (p/n MB-082) for 30 min at RT. Predicted/Observed size: 52 kDa for PDCD.

References

- Sikorski et al. A high-throughput pipeline for validation of antibodies. *Nature Methods* (2018)
- Kim et al. Activation of miR-21-Regulated Pathways in Immune Aging Selects against Signatures Characteristic of Memory T Cells. *Cell Reports* (2018)
- Ng PKS et al. Systematic functional annotation of somatic mutations in cancer. *Cancer Cell.* (2018)
- Hill SM et al. Context specificity in causal signaling networks revealed by phosphoprotein profiling. *Cell Syst.* (2017)
- Kudinov A et al. Msi2 Regulates the Aggressiveness of Non Small Cell Lung Cancer (NSCLC). *Proc Natl Acad Sci USA.* (2016)
- Liwak-Muir et al. ERK8 is a novel HuR kinase that regulates tumour suppressor PDCD4 through a miR-21 dependent mechanism. *Oncotarget* (2016)
- Sharma SB et al. MicroRNAs 206 and 21 Cooperate To Promote RAS–Extracellular Signal-Regulated Kinase Signaling by Suppressing the Translation of RASA1 and SPRED1. *Mol Cell Biol.* (2014)
- Yang, CH et al. The curcumin analog EF24 targets NF-κB and miRNA-21, and has potent anticancer activity in vitro and in vivo. *PloS One* (2014)
- Shen, F et al. MicroRNA-21 Down-regulates Rb1 Expression by Targeting PDCD4 in Retinoblastoma. *Journal of Cancer* (2014)
- Hirata K et al. CD44 variant 9 expression in primary early gastric cancer as a predictive marker for recurrence. *Br J Cancer* (2013)
- Ruan Q et al. The microRNA-21− PDCD4 axis prevents type 1 diabetes by blocking pancreatic β cell death. *Proc Natl Acad Sci USA.* (2011)
- Sheedy FJ et al. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. *Nat Immunol.* (2010)
- Wei, N A. et al. Loss of Programmed cell death 4 (Pdcd4) associates with the progression of ovarian cancer. *Molecular Cancer* (2009)

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