

Datasheet for 200-301-964S**Pdcd4 phospho S457 Antibody****Overview**

Description:	Anti-Pdcd4 pS457 (MOUSE) Monoclonal Antibody - 200-301-964S
Item No.:	200-301-964S
Size:	25 µL
Applications:	ELISA, IHC, WB
Reactivity:	Human, Mouse
Host Species:	Mouse

Product Details

Background:	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:	mouse anti-Pdcd4 pS457 Antibody, 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
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	9G6
Format:	IgG1

Target Details

Gene Name:	PDCD4
Reactivity:	Human, Mouse
PTM Specificity:	Phosphorylation

Immunogen Type:	Conjugated Peptide
Immunogen:	This monoclonal antibody was produced by repeated immunizations with a synthetic phosphopeptide corresponding to residues surrounding Ser457 of the human Pdcd4 protein.
Purity/Specificity:	This product was purified from concentrated tissue culture supernate by Protein A chromatography. 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.
Relevant Links:	• UniProtKB - Q53EL6 • NCBI - 21735596 • GenelD - 27250

Application Details

Tested Applications:	ELISA, IHC, WB
Application Note:	Pdcd4 phospho S457 Antibody is tested by ELISA, immunohistochemistry, and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 62 kDa in size corresponding to phosphorylated Pdcd4 protein by western blotting in the appropriate cell lysate or extract. This phospho-specific monoclonal antibody reacts with human Pdcd4 pS457 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:100,000
IHC:	1:1,000 - 1:5,000
WB:	1:2,000 - 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

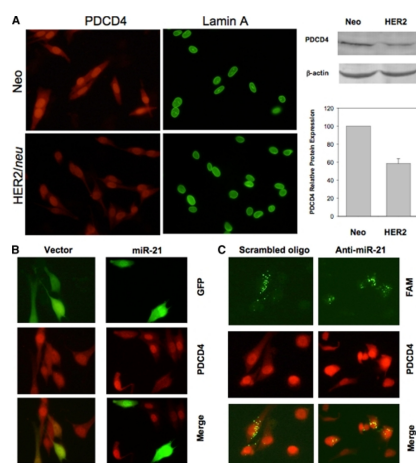
Shipping & Handling

Shipping Condition: Dry Ice

Storage Condition: Store vial at -20° C 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.

Expiration: Expiration date is three (3) months from date of receipt.

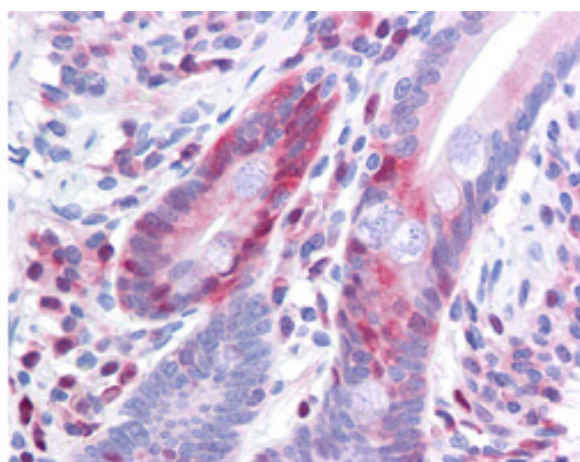
Images



Immunofluorescence Microscopy

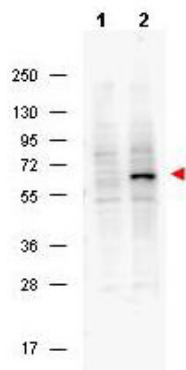
HER2/neu down-regulates PDCD4 expression via miR-21. A, detection of PDCD4 expression in MDA-MB-435/HER2/neu and MDA-MB-435/Neo cells by immunofluorescence microscopy. Nuclear structure protein lamin A represented a positive control in this experiment. B, miR-21 down-regulates PDCD4 in MDA-MB-435/Neo cells. C, anti-miR-21 increased PDCD4 level in MDA-MB-435/HER2/neu. In B and C, cells were first transfected with either miR-21 expression vector or carboxyfluorescein (FAM)-labeled anti-miR-21 and then immunostained with PDCD4 antibody, as described under “Experimental Procedures.”

FIGURE 5. PMID: 19419954



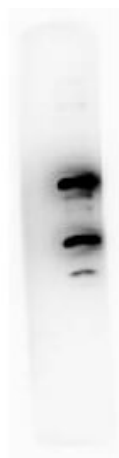
Immunohistochemistry

Rockland Antibody 200-301-964 has been tested in immunohistochemistry, analyzed by an anatomic pathologist and validated for use in IHC applications against formalin-fixed, paraffin-embedded human tissues. The antibody was serially diluted and tested at a range of concentrations on at least 22 different human formalin-fixed, paraffin archival tissues, and positive and negative tissues were scored and compared to the published literature on the expression and function of the gene. A representative image from positively stained small intestine shows the localization of the anti Pdcd4 antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Image provided courtesy of LifeSpan Biosciences, Seattle, WA



Western Blot

Western blot using Rockland's Protein A purified Mouse Monoclonal anti-Pdcd4 pS457 antibody shows detection of phosphorylated Pdcd4 (indicated by arrowhead at ~62 kDa) in NIH-3T3 cells (p/n W10-001-377) after 5 min treatment with 30 ng/mL PDGF [lane 2]. No reactivity is seen for (non-phosphorylated) in unstimulated NIH 3T3 cells (p/n W10-000-358) [lane 1]. The membrane was probed with the primary antibody at a 1:2,000 dilution, overnight at 4° C. For detection HRP conjugated Rb-a-Mouse IgG (p/n 610-4302) was used at a 1:20,000 dilution in blocking buffer (p/n MB-070) for 1 h at 4° C followed by visualization using a Biospectrum® imaging system (UVP).



Western Blot

Western blot using Rockland Immunochemicals Protein A purified Mouse Monoclonal anti-Pdcd4 pS457 antibody against recombinant PDCD4 protein. Membrane was blocked in 1% BSA-TBS-T for 30 min RT and probed with 1° Ab Ms-A-Pdcd4pS457 1:1000 (o/n 4°C in 1% BSA-TBS-T) followed by 2° Ab Peroxidase Conjugated Rabbit anti-Ms CUST10M Lot# 20121 at 1:40,000 in MB-070 30 min RT. Bands at ~62 kD and ~32 kD were detected.

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.