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p16INK4a Monoclonal Antibody (1D7D2)

Catalog Number MA5-17054

Product data sheet

Details

Size	100 µL
Host/Isotope	Mouse / IgG1
Class	Monoclonal
Type	Antibody
Clone	1D7D2
Immunogen	Purified recombinant fragment of human CDKN2A (amino acids: 1-156) expressed in E. Coli.
Conjugate	Unconjugated
Form	Liquid
Concentration	Conc. Not Determined
Storage buffer	ascites
Contains	0.03% sodium azide
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

Species Reactivity

Species reactivity	Human
Published species	Human

Tested Applications	Dilution *
ELISA (ELISA)	1:10,000
Flow Cytometry (Flow)	1:200-1:400
Immunohistochemistry (Paraffin) (IHC (P))	1:200-1:1,000
Western Blot (WB)	1:500-1:2,000
Immunocytochemistry (ICC/IF)	1:100

Published Applications

Immunohistochemistry (IHC)	See 2 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

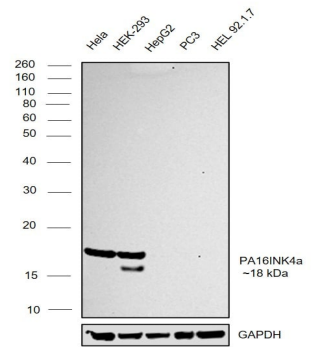
MA5-17054 targets CDKN2A in FACS, IHC, indirect ELISA, and WB applications and shows reactivity with Human samples. The MA5-17054 immunogen is purified recombinant fragment of human CDKN2A (amino acids: 1-156) expressed in E. Coli. MA5-17054 detects CDKN2A which has a predicted molecular weight of approximately 16.5kDa.

Background/Target Information

This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene.

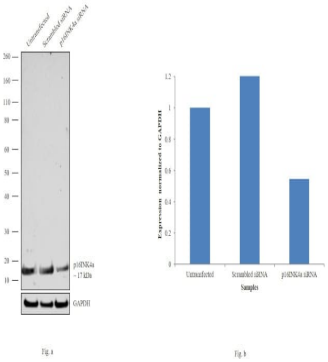
For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization.

Product Images For p16INK4a Monoclonal Antibody (1D7D2)



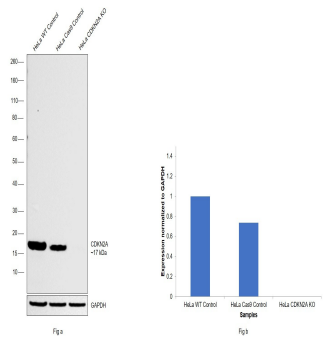
p16INK4a Antibody (MA5-17054) in WB

Western blot was performed using Anti-p16INK4a Monoclonal Antibody (1D7D2) (Product # MA5-17054) and a ~18 kDa band corresponding to CDKN2A was observed across cell lines tested. Whole cell extracts (30 µg lysate) of HeLa (Lane 1), HEK-293 (Lane 2), Hep G2 (Lane 3), PC-3 (Lane 4), HEL 92.1.7 (Lane 5) were electrophoresed using NuPAGE™ 12% Bis-Tris Protein Gel (Product # NP0341BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:1000) and detected by chemiluminescence with Goat anti-Mouse IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A28177,1:20000) using the iBright™ FL1500 Imaging System (Product # A44115). Chemiluminescent detection was performed using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Product # 34580).



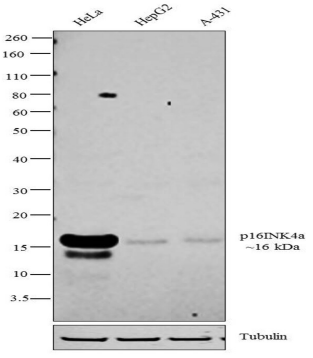
p16INK4a Antibody (MA5-17054)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with p16INK4a siRNA and reduction of signal was observed in Western Blot using p16INK4a Monoclonal Antibody (1D7D2) (Product # MA5-17054). {KD}



p16INK4a Antibody (MA5-17054)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in CDKN2A KO cell line compared to control cell line using Anti-p16INK4a Monoclonal Antibody (1D7D2)(Product # MA5-17054). {KO}



p16INK4a Antibody (MA5-17054) in WB

Western blot analysis was performed on modified whole cell extracts (1% SDS) (30 µg lysate) of HeLa (Lane 1), Hep G2 (Lane 2) and A-431 (Lane 3). The blot was probed with Anti- p16INK4a Monoclonal Antibody (Product # MA5-17054, 1:1000 dilution) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/ml, 1:4000 dilution). A 16 kDa band corresponding to p16INK4a was observed across the cell lines tested.

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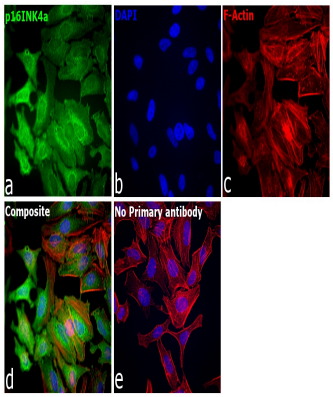
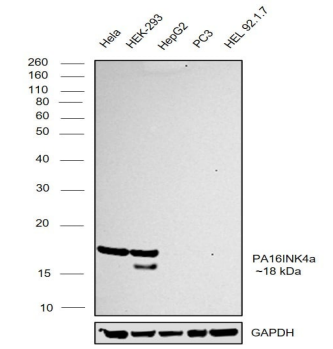


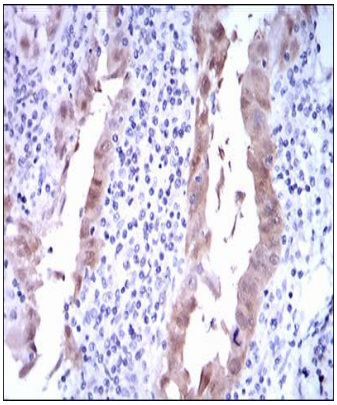
p16INK4a Antibody (MA5-17054) in ICC/IF

Immunofluorescence analysis of CDKN2A was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with p16INK4a Monoclonal Antibody (1D7D2) (Product # MA5-17054) at 1:100 in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32766), (1:2000), for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b:Blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing nucleus and cytoplasm localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 40X. magnification.



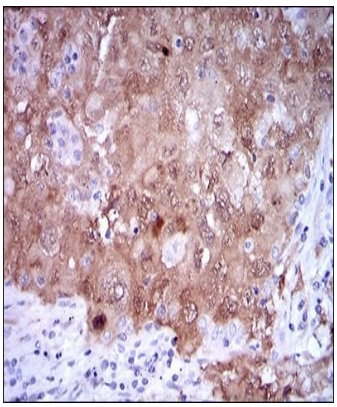
p16INK4a Antibody (MA5-17054)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell lines owing to their inherent genetic constitution. Relative expression of CDKN2A was observed as higher expression in HeLa and HEK-293 compared to other cell lines using Anti-p16INK4a Monoclonal Antibody (1D7D2) (Product # MA5-17054) in Western Blot. {RE}



p16INK4a Antibody (MA5-17054) in IHC (P)

Immunohistochemical analysis of paraffin-embedded endometrial cancer tissues using CDKN2A monoclonal antibody (Product # MA5-17054) followed with DAB staining.



p16INK4a Antibody (MA5-17054) in IHC (P)

Immunohistochemical analysis of paraffin-embedded lung cancer tissues using CDKN2A monoclonal antibody (Product # MA5-17054) followed with DAB staining.

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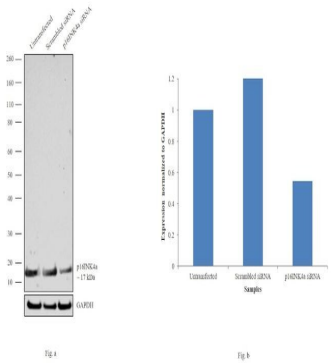
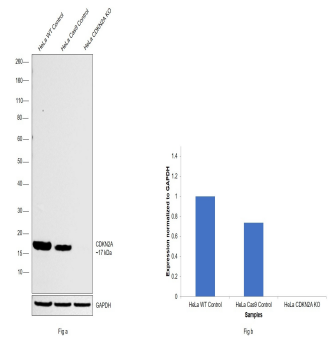


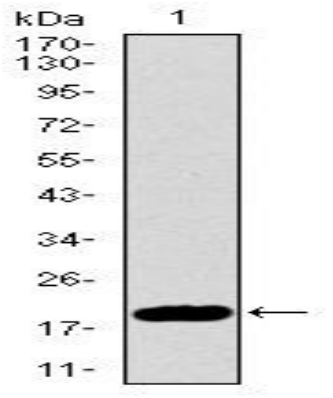
p16INK4a Antibody (MA5-17054) in WB

Knockdown of p16INK4a was achieved by transfecting Caco-2 with p16INK4a specific siRNAs (Silencer® select Product # s216). Western blot analysis (Fig. a) was performed using whole cell extracts from the p16INK4a knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blots were probed with p16INK4a Monoclonal Antibody (1D7D2) (Product # MA5-17054, 1:2000 dilution) and Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/ml 1: 4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to p16INK4a.



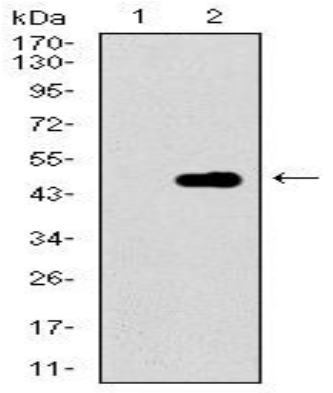
p16INK4a Antibody (MA5-17054) in WB

Knockout of CDKN2A was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042) (Assay ID CRISPR697306_LV) and LentiArray Cas9 Lentivirus (Product # A32064). Western blot analysis of CDKN2A was performed by loading 30 µg of HeLa wild type (Lane 1), HeLa CAS9 (Lane 2), HeLa CDKN2A KO (Lane 3) whole cell extracts. The blot was probed with Anti-p16INK4a Monoclonal Antibody (1D7D2)(Product # MA5-17054) using 1:2000 dilution and Goat anti-Mouse IgG (H+L), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A28177). Loss of signal upon CRISPR mediated knockout (KO) using the LentiArray™ CRISPR product line confirms that antibody is specific to CDKN2A.



p16INK4a Antibody (MA5-17054) in WB

Western blot analysis of CDKN2A using a CDKN2A monoclonal antibody (Product # MA5-17054) against a human CDKN2A recombinant protein.



p16INK4a Antibody (MA5-17054) in WB

Western blot analysis of CDKN2A using CDKN2A monoclonal antibody (Product # MA5-17054) in HEK293 (1) and CDKN2A (AA: 1-156) human IgG Fc transfected HEK293 (2) cell lysate.

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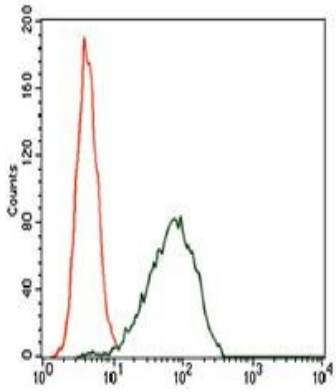

p16INK4a Antibody (MA5-17054) in Flow

Flow cytometric analysis of HEK293 cells using CDKN2A monoclonal antibody (Product # MA5-17054) (green) and negative control (red).

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PubMed References For p16INK4a Monoclonal Antibody (1D7D2)

2 Immunohistochemistry References

Species / Dilution	Summary
Human / 1:50	MA5-17054 was used in Immunohistochemistry-immunofluorescence to find that age itself has a negative impact on SMCs in the ascending aortic wall, whereby SMCs switched from the contractile phenotype to maladaptive synthetic or senescent states with increased age. Frontiers in cardiovascular medicine (2023; 10:) **"Age-dependent phenotypic modulation of smooth muscle cells in the normal ascending aorta."** Author(s):Balint B,Bernstorff IGL,Schwab T,Schäfers HJ PubMed Article URL:http://dx.doi.org/10.3389/fcvm.2023.1114355
Human / 1:100	MA5-17054 was used in Immunohistochemistry to discuss some of the main features of giant benign phyllodes tumor along with the diagnostic and therapeutic difficulties. Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie (2022; 62: 1035) **"Diagnostic difficulties in giant benign phyllodes tumor."** Author(s):Matei RA,Mehedinu-Ionescu M,Paitici ,Georgescu EF,Donoiu A,Ghemigian AM,Popescu M,Totolici BD,Neamu C,Mogoant S PubMed Article URL:http://dx.doi.org/10.47162/RJME.62.4.16

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