

MDM2 Monoclonal Antibody (IF2)

Catalog Number 33-7100

Product data sheet

Details	
Size	50 µg
Host/Isotope	Mouse / IgG2b, kappa
Class	Monoclonal
Type	Antibody
Clone	IF2
Immunogen	Synthetic peptide derived from the n-terminal region of human MDM2
Conjugate	Unconjugated
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS, pH 7.4, with 1mg/mL BSA, 30% glycerol
Contains	0.05% sodium azide
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles

Species Reactivity	
Species reactivity	Human
Published species	Rat, Bacteria, Human, Not Applicable, Guinea pig
Tested Applications	
Immunocytochemistry (ICC)	1:25-1:200
Immunofluorescence (IF)	1:25-1:200
Immunohistochemistry (IHC)	1:25-1:200
Immunoprecipitation (IP)	1-5 µg/mL
Western Blot (WB)	0.5-2 µg/mL
Published Applications	
Miscellaneous PubMed (MISC)	See 5 publications below
Immunohistochemistry (Paraffin) (IHC (P))	See 6 publications below
Immunohistochemistry (IHC)	See 8 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

This antibody recognizes the ~90 kDa (apparent MW) MDM2 protein. Also recognizes isoforms at ~57 and ~74/76 kDa. The epitope recognized by this antibody is located within amino acids 26-169 of the human protein. Positive controls: OsA-CL, MCF-7, HeLa cell lysates. HOS cells can be used for negative control. Staining of formalin fixed, paraffin embedded tissue requires heat induced epitope retrieval pretreatment.

Background/Target Information

MDM2 is a ubiquitin ligase for p53 and plays a central role in regulation of the stability of p53. MDM2 is located on chromosome 12 on the q arm. Akt-mediated phosphorylation of MDM2 at Ser166 and Ser186 increases its interaction with p300, allowing MDM2-mediated ubiquitination and degradation of p53. Phosphorylation of MDM2 also blocks its binding to p19ARF, increasing the degradation of p53. MDM2 has also been shown to negatively regulate p53 function. MDM2 binds and inhibits transactivation role played by p53 and overexpression of MDM2 can result in the inactivation of p53 and decrease its tumor suppressor function. Another process by which MDM2 can inactivate p53 is by degrading p53 as the protein also possesses E3 ubiquitin ligase activity. Further, MDM2 plays important roles in apoptosis and cell cycle. MDM2 is over expressed in a wide range of human malignancies including soft tissue carcinomas and breast cancer. In addition to p53, MDM2 is involved in processes of cell cycle, apoptosis, and tumorigenesis through interactions with proteins that include retinoblastoma 1 and ribosomal protein L5. More than 40 different alternatively spliced transcript variants of MDM2 have been isolated from both tumor and normal tissues.

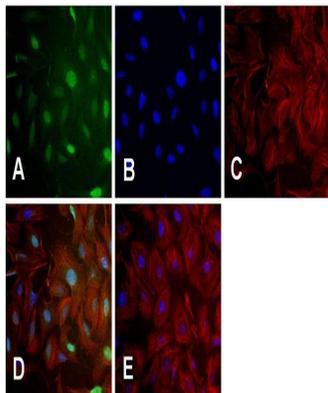
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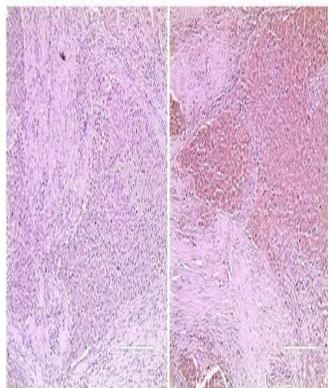
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Product Images For MDM2 Monoclonal Antibody (IF2)



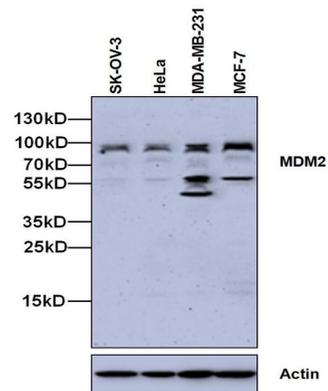
MDM2 Antibody (33-7100) in IF

Immunofluorescence analysis of MDM2 in subconfluent U2OS cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 3% BSA for 15 minutes at room temperature. The cells were probed with a MDM2 Mouse Monoclonal Antibody (Product # 33-7100) at 1.5 µg/mL for 1 hour at room temperature and then labeled with a Goat anti-Mouse IgG (H+L) Superclonal Secondary Antibody, Alexa Fluor 488 conjugate (Product # A28175) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with Hoechst Dye. F-actin (Panel c: red) was stained with DyLight 554 Phalloidin (Product # 21834). Panel d is a merged image showing predominantly nuclear localization. Panel e shows no primary antibody control. The images were captured at 20X magnification.



MDM2 Antibody (33-7100) in IHC

Immunohistochemistry analysis of MDM2 showing staining in the cytoplasm and nucleus of paraffin-embedded human breast carcinoma (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0) and heated in a 95C water bath for 20 minutes. Following antigen retrieval, tissues were blocked in 10% goat serum in PBS for 30 minutes at room temperature and quenched with Peroxide Suppressor (Product # 35000) for 30 minutes. Tissues were then probed with a MDM2 monoclonal antibody (Product # 33-7100) at a dilution of 40 µg/mL in blocking buffer for 1 hour at room temperature. Tissues were washed extensively in PBST and detection was performed using the SuperPicture HRP Polymer Detection Kit (Product # 87-8963) and DAB substrate (Product # 34002). Tissues were counterstained with hematoxylin (Product # TA-125-MH) and dehydrated with ethanol and xylene to prep for mounting.



MDM2 Antibody (33-7100) in WB

Western blot analysis of MDM2 was performed by loading 30 µg of the indicated whole cell lysates and 5 µL of PageRuler Plus Prestained Protein Ladder (Product # 26619) per well onto a Novex 4-20% Tris-Glycine polyacrylamide gel (Product # WT4202BOX). Proteins were transferred to a nitrocellulose membrane using the G2 Blotter (Product # 62288), and blocked with 5% Milk in TBST for 1 hour at room temperature. MDM2 was detected at ~57, ~75, and ~90 kDa using a MDM2 monoclonal antibody (Product # 33-7100) at a dilution of 2.5 µg/mL in 5% Milk in TBST overnight at 4C on a rocking platform, followed by a Goat anti-Mouse IgG (H+L) Superclonal Secondary Antibody, HRP conjugate (Product # A28177) at a dilution of 1:1000 for at least 30 minutes at room temperature. Chemiluminescent detection was performed using SuperSignal Pico substrate (Product # 34078) and the myECL Imager (Product # 62236).

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PubMed References For MDM2 Monoclonal Antibody (IF2)

5 Miscellaneous PubMed References

Species / Dilution	Summary
Human / 1:100	33-7100 was used in immunohistochemistry - paraffin section to explore the use of MDM2 and CDK4 for the histological diagnosis of low-grade osteosarcoma Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc (Sep 2010; 23: 1279) "Immunohistochemical analysis of MDM2 and CDK4 distinguishes low-grade osteosarcoma from benign mimics." Author(s):Yoshida A,Ushiku T,Motoi T,Shibata T,Beppu Y,Fukayama M,Tsuda H PubMed Article URL: http://dx.doi.org/10.1038/modpathol.2010.124
Human / Not Cited	33-7100 was used in immunohistochemistry to investigate the frequency and contribution of HMGA2, CDK4, and JUN to dedifferentiated liposarcomas and atypical lipomatous tumors/well-differentiated liposarcomas. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc (Nov 2015; 28: 1404) "Prognostic value of HMGA2, CDK4, and JUN amplification in well-differentiated and dedifferentiated liposarcomas." Author(s):Saâda-Bouziid E,Burel-Vandenbos F,Ranchère-Vince D,Birtwisle-Peyrottes I,Chetaille B,Bouvier C,Château MC,Peoc'h M,Battistella M,Bazin A,Gal J,Michiels JF,Coindre JM,Pedeutour F,Bianchini L PubMed Article URL: http://dx.doi.org/10.1038/modpathol.2015.96
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6 Immunohistochemistry (Paraffin) References

Species / Dilution	Summary
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Not Applicable / 1:100	33-7100 was used in immunohistochemistry - paraffin section to assess if immunostaining for PPAR-gamma distinguishes dedifferentiated liposarcoma from other retroperitoneal sarcomas Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc (May 2008; 21: 517) "Immunostaining for peroxisome proliferator gamma distinguishes dedifferentiated liposarcoma from other retroperitoneal sarcomas." Author(s):Horvai AE,Schaefer JT,Nakakura EK,O'Donnell RJ PubMed Article URL: http://dx.doi.org/10.1038/modpathol.3801017

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8 Immunohistochemistry References

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33-7100 was used in immunohistochemistry to discuss the properties of desmoid tumors

JOP : Journal of the pancreas (May 2008; 9: 339)

"Sporadic desmoid tumor. An exceptional cause of cystic pancreatic lesion."

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Author(s):Nahal A,Meterissian S

PubMed Article URL:<http://dx.doi.org/10.1097/COC.0b013e31818c0926>

Not Applicable / Not Cited

Human / 1:100

Human / Not Cited

Not Applicable / Not Cited

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