abcam

Product datasheet

Anti-MDM2 antibody [2A10] ab16895

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Overview

Product name	Anti-MDM2 antibody [2A10]
Description	Mouse monoclonal [2A10] to MDM2
Host species	Mouse
Specificity	Recognizes the ~90 kDa (apparent MW) MDM2 protein in A549 cells.
Tested applications	**Suitable for:** ICC, WB, IHC-P, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Full length protein corresponding to MDM2 aa 250-350. Database link: **Q00987-1**
Epitope	Within amino acids 294-339.
Positive control	A549 cell lysates. HeLa cells
General notes	This product was changed from ascites to tissue culture supernatant on 17 May 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at 4°C (stable for up to 12 months). Do Not Freeze.
Storage buffer	pH: 7.40 Preservative: 0.05% Sodium azide Constituents: 0.88% Sodium chloride, Tris glycine
Purity	Tissue culture supernatant
Purification notes	Purified from TCS.

Clonality	Monoclonal
Clone number	2A10
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab16895 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC		Use at an assay dependent concentration.
WB	★★★★☆ (3)	Use at an assay dependent concentration. Detects a band of approximately 90 kDa (predicted molecular weight: 55 kDa).
IHC-P		Use at an assay dependent concentration.
ICC/IF	★★★★☆ (1)	Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Target

Function E3 ubiquitin-protein ligase that mediates ubiquitination of p53/TP53, leading to its degradation by the proteasome. Inhibits p53/TP53- and p73/TP73-mediated cell cycle arrest and apoptosis by binding its transcriptional activation domain. Also acts as an ubiquitin ligase E3 toward itself and ARRB1. Permits the nuclear export of p53/TP53. Promotes proteasome-dependent ubiquitin-independent degradation of retinoblastoma RB1 protein. Inhibits DAXX-mediated apoptosis by inducing its ubiquitination and degradation. Component of the TRIM28/KAP1-MDM2-p53/TP53 complex involved in stabilizing p53/TP53. Also component of the TRIM28/KAP1-ERBB4-MDM2 complex which links growth factor and DNA damage response pathways.

Tissue specificity Ubiquitous. Isoform Mdm2-A, isoform Mdm2-B, isoform Mdm2-C, isoform Mdm2-D, isoform Mdm2-E, isoform Mdm2-F and isoform Mdm2-G are observed in a range of cancers but absent in normal tissues.

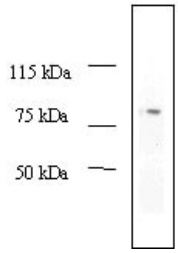
Involvement in disease Note=Seems to be amplified in certain tumors (including soft tissue sarcomas, osteosarcomas and gliomas). A higher frequency of splice variants lacking p53 binding domain sequences was found in late-stage and high-grade ovarian and bladder carcinomas. Four of the splice variants show loss of p53 binding.

Sequence similarities Belongs to the MDM2/MDM4 family.
Contains 1 RanBP2-type zinc finger.
Contains 1 RING-type zinc finger.
Contains 1 SWIB domain.

Domain Region I is sufficient for binding p53 and inhibiting its G1 arrest and apoptosis functions. It also binds p73 and E2F1. Region II contains most of a central acidic region required for interaction with ribosomal protein L5 and a putative C4-type zinc finger. The RING finger domain which

	coordinates two molecules of zinc interacts specifically with RNA whether or not zinc is present and mediates the heterooligomerization with MDM4. It is also essential for its ubiquitin ligase E3 activity toward p53 and itself.
Post-translational modifications	Phosphorylated in response to ionizing radiation in an ATM-dependent manner. Auto-ubiquitinated; which leads to proteasomal degradation. Deubiquitinated by USP2 leads to its accumulation and increases deubiquitinilation and degradation of p53/TP53. Deubiquitinated by USP7; leading to stabilize it.
Cellular localization	Nucleus > nucleoplasm. Cytoplasm. Nucleus > nucleolus. Expressed predominantly in the nucleoplasm. Interaction with ARF(P14) results in the localization of both proteins to the nucleolus. The nucleolar localization signals in both ARF(P14) and MDM2 may be necessary to allow efficient nucleolar localization of both proteins. Colocalizes with RASSF1 isoform A in the nucleus.

Images



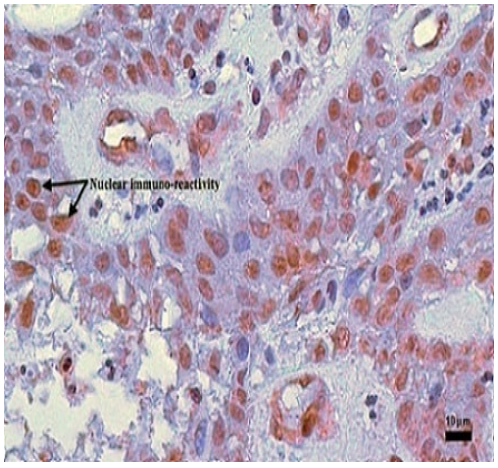
Western blot - Anti-MDM2 antibody [2A10] (ab16895)

Anti-MDM2 antibody [2A10] (ab16895) at 2 µg/ml + A549 whole cell lysate

Predicted band size: 55 kDa

Detection: chemiluminescence.

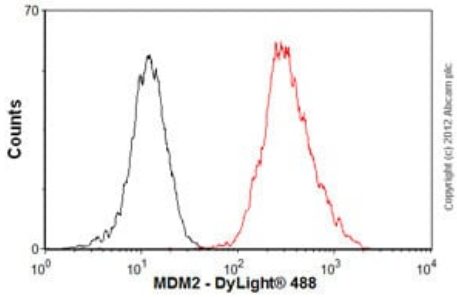
This image was generated using the ascites version of the product.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MDM2 antibody [2A10] (ab16895)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of ameloblastoma tissue sections labeling MDM2 with ab16895. The sections were de-paraffinized, hydrated and then rinsed in phosphate-buffered solution (PBS). They were immersed in heat-induced epitope retrieval citrate buffer of concentration 15mMol and pH 6.0, diluted 1/10 with distilled water and incubated at 95ºC for 10 minutes. They were then placed in fresh citrate, cooled in water for 20 minutes and then rinsed in PBS for 6 minutes. Peroxidase blocking reagent was added to each section for 5 minutes, and the sections were rinsed in 0.1% PBS for 6 minutes. The specimen were incubated for 30 minutes with 1/100 dilution of Anti-MDM2 antibody [2A10] (ab16895), then rinsed with PBS, followed by incubation with undiluted HRP for 20 minutes. 1ml of diaminobenzidine solution was added to cover the specimen, followed by incubation in a humidity chamber for 15 minutes. The sections were then immersed in aqueous haematoxylin and rinsed in distilled water for 5 minutes. The tissue was dehydrated and subsequently rinsed with xylene. Distyrene plasticizer in xylene mounting fluid was then applied, and a cover slip placed. Hematoxylin and eosin staining.

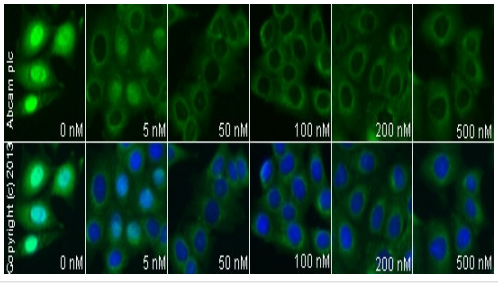
This image was generated using the ascites version of the product.



Flow Cytometry - Anti-MDM2 antibody [2A10] (ab16895)

Overlay histogram showing HeLa cells stained with **ab16895** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum (**ab7481**) / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab16895, 0.5µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (**ab91361**, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

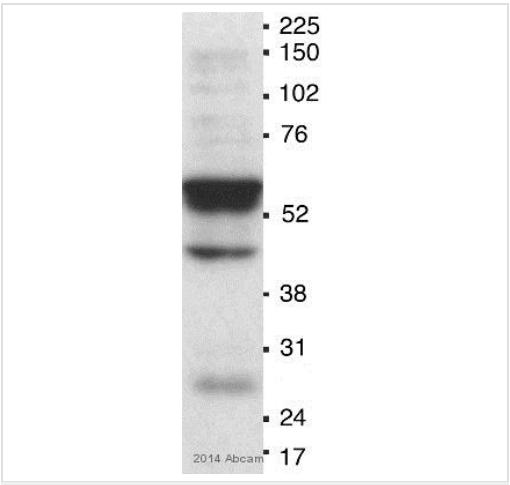
This image was generated using the ascites version of the product.



Immunocytochemistry/ Immunofluorescence - Anti-MDM2 antibody [2A10] (ab16895)

ab16895 staining MDM2 in MCF7 cells treated with progesterone (**ab141252**), by ICC/IF. Decrease in MDM2 expression correlates with increased concentration of progesterone, as described in literature.
The cells were incubated at 37°C for 24 hour in media containing different concentrations of **ab141252** (progesterone) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab16895 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-mouse polyclonal antibody (**ab96879**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

This image was generated using the ascites version of the product.



Western blot - Anti-MDM2 antibody [2A10] (ab16895)

Image is courtesy of an anonymous Abreview

Anti-MDM2 antibody [2A10] (ab16895) at 1/1000 dilution + Mouse Liver lysate at 40 µg with Milk, 2 hours at 25°C at 5 %

Secondary

Donkey anti-mouse IgG (HRP) at 1/10000 dilution

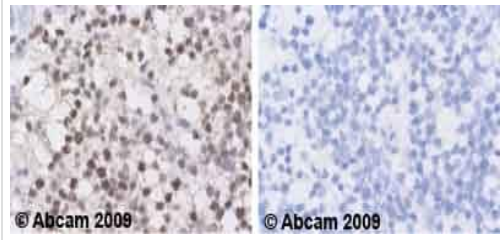
Performed under reducing conditions.

Predicted band size: 55 kDa

Additional bands at: 55 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 minute

This image was generated using the ascites version of the product.



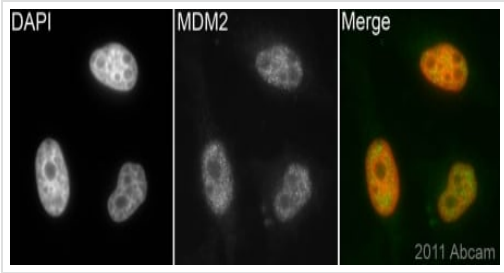
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MDM2 antibody [2A10] (ab16895)

Please note: for manual staining we recommend to optimize primary antibody concentration and incubation time (overnight incubation); amplification may be required.

ab16895 staining Human normal tonsil. Staining is localised to nuclear + cytoplasmic compartments. Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.

Sections were stained using an automated system DAKO Autostainer Plus, at RT: sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffers citrate pH6.1 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 min. They were then blocked with Dako Protein block for 10 min (containing casein 0.25% in PBS) , incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 min. Colorimetric detection was completed with DAB for 5 min. Slides were counterstained with Haematoxylin and coverslipped under DePeX.

This image was generated using the ascites version of the product.



Immunocytochemistry - Anti-MDM2 antibody [2A10] (ab16895)

Image courtesy of an Abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MICB, Canada

ab16895 (1/200) staining MDM2 in assynchronous HeLa cells (green). Cells were fixed with paraformaldehyde, permeabilized with 0.5% Triton X100 and counterstained with DAPI in order to highlight the nucleus (red). Please refer to Abreview for further experimental details.

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