

Product datasheet

Anti-MDM2 antibody [SMP 14] ab3110

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Overview

Product name	Anti-MDM2 antibody [SMP 14]
Description	Mouse monoclonal [SMP 14] to MDM2
Specificity	This antibody also recognises a peptide epitope around Thr-216 of murine MDM2 (when Thr-216 is unphosphorylated) see Zhang & Prives, 2001 for further details. SMP14 also cross reacts with some cytokeratins (6, 14 & 16). This is only a problem when working with certain epithelial cells and not fibroblasts.
Tested applications	Suitable for: Flow Cyt, WB, IHC-Fr, IP, ELISA, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human MDM2 aa 154-167. Cross-linked to KLH using glutaldehyde. Sequence: C-SRPSTSSRRRAISE
Epitope	Amino acids 154 - 167.
Positive control	Breast carcinoma. Human Pancreas cells HeLa cells

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Constituent: PBS Please note that some batches of ab3110 may contain 0.4M arginine. Please contact Scientific Support for further information.
Purity	Protein G purified
Clonality	Monoclonal
Clone number	SMP 14
Myeloma	x63-Ag8.653
Isotype	IgG1
Light chain type	kappa

Applications

Our [Abpromise guarantee](#) covers the use of **ab3110** in the following tested applications.

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

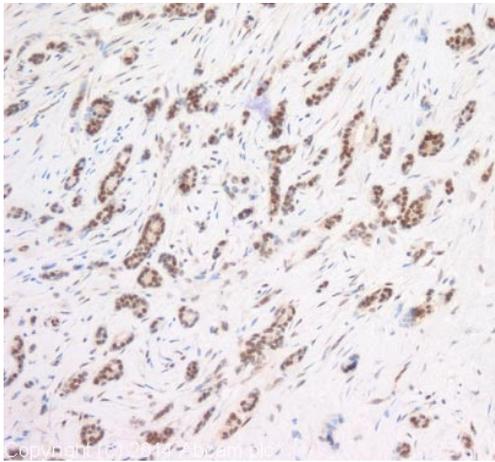
Application	Abreviews	Notes
Flow Cyt		Use 1µg for 10 ⁶ cells.
WB	★★★★☆	Use at an assay dependent concentration. Predicted molecular weight: 55 kDa.
IHC-Fr		Use at an assay dependent concentration.
IP	★★★★☆	Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.
IHC-P	★★★★★	Use a concentration of 1 - 2 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

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 deubiquitination 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.

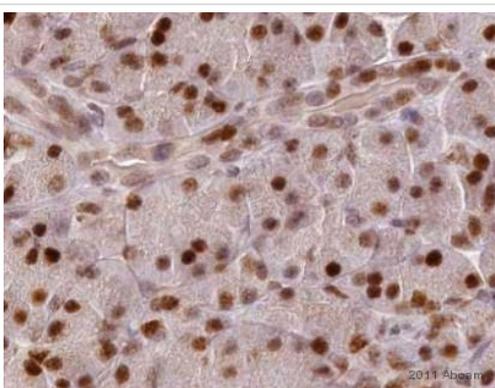
Anti-MDM2 antibody [SMP 14] images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MDM2 antibody [SMP 14] (ab3110)

IHC image of MDM2 staining in human breast adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab3110, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

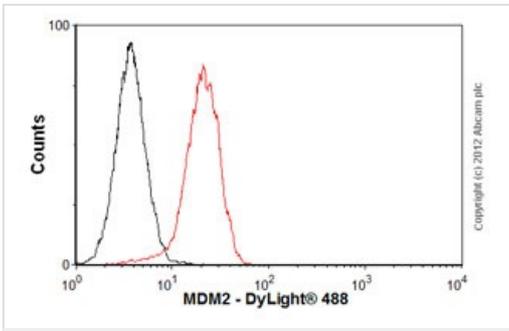
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - MDM2 antibody [SMP 14] (ab3110)

This image is courtesy of an Anonymous Abreview.

[ab3110](#) staining MDM2 in human pancreas tissue ([ab29816](#)) by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded tissue sections). The sections were fixed in paraformaldehyde and subjected to heat-mediated antigen retrieval in citric buffer pH 6.0, prior to blocking with 10% serum for 1 hour at 12°C. The primary antibody was diluted 1/100 and incubated with the sample for 12 hours at 4°C. An HRP-conjugated rabbit anti-mouse polyclonal was used as the secondary antibody, diluted 1/200.



Flow Cytometry - Anti-MDM2 antibody [SMP 14] (ab3110)

Overlay histogram showing HeLa cells stained with **ab3110** (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 (**ab3110**, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

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