abcam

Product datasheet

Anti-MDM2 (phospho \$166) antibody [EPR1450(2)] - BSA and Azide free ab249560

Recombinant

RabMAb

6 Images

Overview

Product name Anti-MDM2 (phospho S166) antibody [EPR1450(2)] - BSA and Azide free

Description Rabbit monoclonal [EPR1450(2)] to MDM2 (phospho S166) - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Dot blot, IHC-P, WB

Unsuitable for: Flow Cyt,ICC/IF or IP

Species reactivity Reacts with: Human

Predicted to work with: Rat
Does not react with: Mouse

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

General notes ab249560 is the carrier-free version of <u>ab170880</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

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Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

PurityAffinity purifiedClonalityMonoclonalClone numberEPR1450(2)

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab249560 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
Dot blot		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 55 kDa.

Application notes

Is unsuitable for Flow Cyt,ICC/IF or IP.

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.

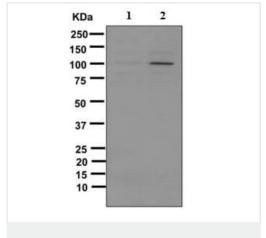
Auto-ubiquitinated; which leads to proteasomal degradation. Deubiquitinated by USP2 leads to its accumulation and increases deubiquitinilation and degradation of p53/TP53. Deubiquitinated accumulation of p53/TP53. Deubiquitinated accumulation and degradation of p53/TP53. Deubiquitinated accumulation accumulat

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 (phospho S166) antibody [EPR1450(2)] - BSA and Azide free (ab249560) **All lanes :** Anti-MDM2 (phospho S166) antibody [EPR1450(2)] (**ab170880**) at 1/50000 dilution

Lane 1: MCF7 cell lysate

Lane 2: MCF7 cell lysate treated with IGF-1

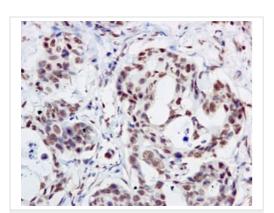
Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP labelled goat anti-rabbit lgG at 1/2000 dilution

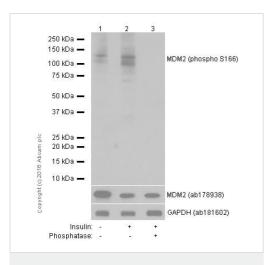
Predicted band size: 55 kDa

This data was developed using **ab170880**, the same antibody clone in a different buffer formulation.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MDM2 (phospho S166) antibody [EPR1450(2)] - BSA and Azide free (ab249560)

This data was developed using <u>ab170880</u>, the same antibody clone in a different buffer formulation.lmmunohistochemical analysis of paraffin-embedded human gastric carcinoma tissue labeling MDM2 (phospho S166) using <u>ab170880</u> at a 1/50 dilution. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-MDM2 (phospho S166) antibody [EPR1450(2)] - BSA and Azide free (ab249560)

All lanes : Anti-MDM2 (phospho S166) antibody [EPR1450(2)] (ab170880) at 1/100000 dilution

Lane 1: A549 (Human lung carcinoma epithelial cell) whole cell lysate

Lane 2: A549 (Human lung carcinoma epithelial cell) treated with insulin at 1ug/ml for 150 minutes. Whole cell lysate

Lane 3: A549 (Human lung carcinoma epithelial cell) treated with insulin at 1ug/ml for 150 minutes. Whole cell lysate. Then the membrane was incubated with phosphatase

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 55 kDa **Observed band size:** 90-140 kDa

Exposure time: 5 seconds

This data was developed using <u>ab170880</u>, the same antibody clone in a different buffer formulation.

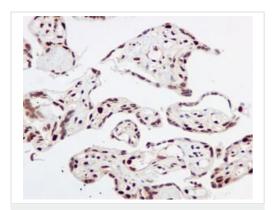
Blocking and dilution buffer: 2% BSA/TBST.

The molecular weight is the same with the one from this paper PMID: 25392082.

1 2 5ng
1ng
0.1ng
0.01ng

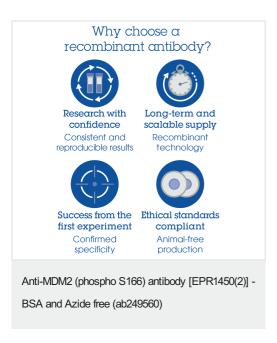
Dot Blot - Anti-MDM2 (phospho S166) antibody [EPR1450(2)] - BSA and Azide free (ab249560)

This data was developed using <u>ab170880</u>, the same antibody clone in a different buffer formulation.Dot blot analysis of MDM2 (phospho S166) phospho peptide (Lane 1), MDM2 non-phospho peptide (Lane 2), labeling MDM2 (phospho S166) with <u>ab170880</u> at a dilution of 1/1000. Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) was used as the secondary antibody at a dilution of 1/100000. Blocking and dilution buffer: 5% NFDM/TBST. Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MDM2 (phospho S166) antibody [EPR1450(2)] - BSA and Azide free (ab249560)

This data was developed using <u>ab170880</u>, the same antibody clone in a different buffer formulation.lmmunohistochemical analysis of paraffin-embedded human placenta tissue labeling MDM2 (phospho S166) using <u>ab170880</u> at a 1/50 dilution. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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