Anti-MDM2 Antibody [A10E11-R]

HA601310

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Monkey
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 55 kDa
Clone number:	A10E11-R

Description: Mouse double minute 2 homolog (MDM2) also known as E3 ubiquitin-protein ligase Mdm2 is a protein that in humans is encoded by the MDM2 gene. Mdm2 is an important negative regulator of the p53 tumor suppressor. Mdm2 protein functions both as an E3 ubiquitin ligase that recognizes the N-terminal trans-activation domain (TAD) of the p53 tumor suppressor and as an inhibitor of p53 transcriptional activation.

Immunogen: Recombinant protein within human MDM2 aa 1-491 / 491.

Positive control: U-2 OS treated with 10μM NUTLIN 3A for 24 hours cell lysate, HepG2 cell lysate, COS-1cell lysate, U-2 OS cells treated with 10μM NUTLIN 3A for 24 hours, human embryonal carcinoma tissue, human testis tissue.

Subcellular location: Nucleus, nucleoplasm, nucleolus, Cytoplasm.

Database links: SwissProt: Q00987 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:500
IHC-P	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

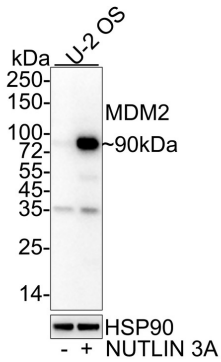

Fig1: Western blot analysis of MDM2 on different lysates with Mouse anti-MDM2 antibody (HA601310) at 1/1,000 dilution.

Lane 1: U-2 OS cell lysate
Lane 2: U-2 OS treated with 10μM NUTLIN 3A for 24 hours cell lysate

Lysates/proteins at 15 μg/Lane.

Predicted band size: 55 kDa
Observed band size: 90 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601310) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

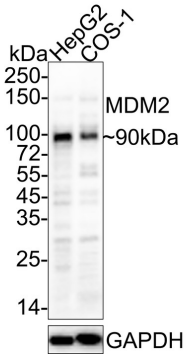

Fig2: Western blot analysis of MDM2 on different lysates with Mouse anti-MDM2 antibody (HA601310) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: COS-1cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 55 kDa
Observed band size: 90 kDa

Exposure time: 46 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601310) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

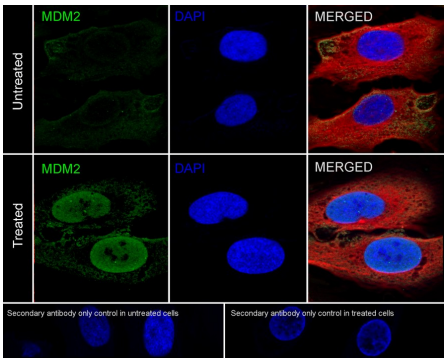

Fig3: Immunocytochemistry analysis of U-2 OS cells treated with or without 10μM NUTLIN 3A for 24 hours labeling MDM2 with Mouse anti-MDM2 antibody (HA601310) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-MDM2 antibody (HA601310) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

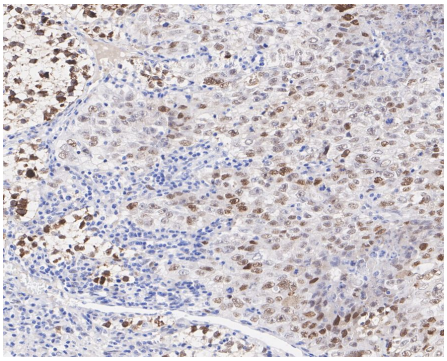

Fig4: Immunohistochemical analysis of paraffin-embedded human embryonal carcinoma tissue with Mouse anti-MDM2 antibody (HA601310) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601310) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

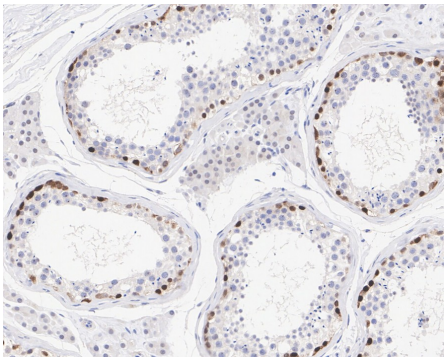

Fig5: Immunohistochemical analysis of paraffin-embedded human testis tissue with Mouse anti-MDM2 antibody (HA601310) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601310) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Konopleva M et al. MDM2 inhibition: an important step forward in cancer therapy. Leukemia. 2020 Nov
2. Klein AM et al. The roles and regulation of MDM2 and MDMX: it is not just about p53. Genes Dev. 2021 May