abcam

Product datasheet

Anti-53BP1 antibody [EPR2172(2)] ab175933





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Overview

Product name Anti-53BP1 antibody [EPR2172(2)]

Description Rabbit monoclonal [EPR2172(2)] to 53BP1

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HepG2 and HeLa cell lysate and human fetal heart and fetal brain tissue lysates, mouse

heart and rat heart tissue lysates. IHC-P: human colon, liver carcinoma and tonsil, mouse and rat

liver tissues. ICC/IF: HepG2 cells.

General notes 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**.

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 Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR2172(2)

Isotype lgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab175933 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
WB		1/1000 - 1/5000. Detects a band of approximately 450 kDa (predicted molecular weight: 214 kDa).
IHC-P		1/60 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF	**** (1)	1/100 - 1/250.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target

Function May have a role in checkpoint signaling during mitosis. Enhances TP53-mediated transcriptional

activation. Plays a role in the response to DNA damage.

Involvement in diseaseNote=A chromosomal aberration involving TP53BP1 is found in a form of myeloproliferative

disorder chronic with eosinophilia. Translocation t(5;15)(q33;q22) with PDGFRB creating a

TP53BP1-PDGFRB fusion protein.

Sequence similarities Contains 2 BRCT domains.

Post-translational modifications

Asymmetrically dimethylated on Arg residues by PRMT1. Methylation is required for DNA binding. Phosphorylated at basal level in the absence of DNA damage. Hyper-phosphorylated in an ATM-

dependent manner in response to DNA damage induced by ionizing radiation. Hyperphosphorylated in an ATR-dependent manner in response to DNA damage induced by UV

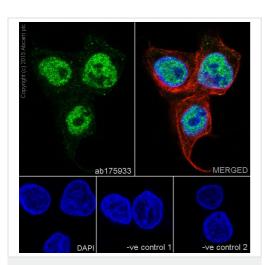
irradiation.

Cellular localization Nucleus. Chromosome > centromere > kinetochore. Associated with kinetochores. Both nuclear

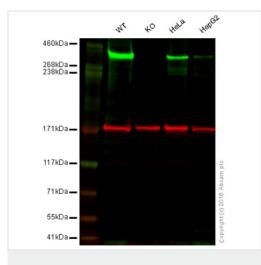
and cytoplasmic in some cells. Recruited to sites of DNA damage, such as double stand breaks.

 $\label{thm:methylation} \mbox{Methylation of histone H4 at 'Lys-20' is required for efficient localization to double strand breaks.}$

Images



Immunocytochemistry/ Immunofluorescence - Anti-53BP1 antibody [EPR2172(2)] (ab175933)



Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling 53BP1 with purified ab175933 at 1/200. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse lgG (1/1000) were also used.

Control 1: primary antibody (1/200) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000).

Lane 1: Wild-type HAP1 cell lysate (20 µg)

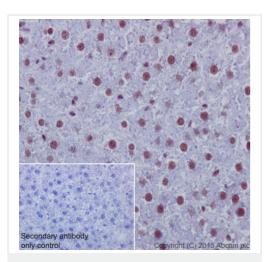
Lane 2: 53BP1 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (40 µg)

Lane 4: HepG2 cell lysate (40 µg)

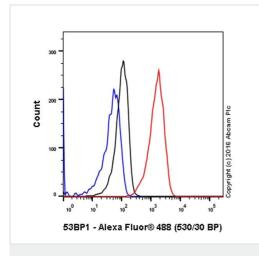
Lanes 1 - 4: Merged signal (red and green). Green - ab175933 observed at 350 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

ab175933 was shown to specifically react with 53BP1 when 53BP1 knockout samples were used. Wild-type and 53BP1 knockout samples were subjected to SDS-PAGE. ab175933 and <u>ab18058</u> (loading control to Vinculin) were diluted 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-53BP1 antibody
[EPR2172(2)] (ab175933)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue labelling 53BP1 with purified ab175933 at a dilution of 1/60. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

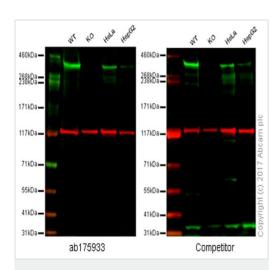


Flow Cytometry (Intracellular) - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

ab175933 staining 53BP1in the human cell lineHepG2 (human hepatocellular carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/30. A goat anti rabbit lgG (Alexa Fluor[®] 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)



Lane 2: 53BP1 knockout HAP1 cell lysate (20 µg)

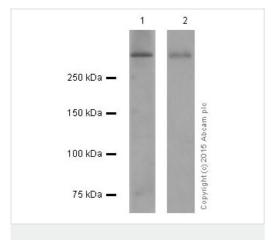
Lane 3: HeLa cell lysate (40 µg)

Lane 4: HepG2 cell lysate (40 µg)

Lanes 1 - 4: Merged signal (red and green).

Green - Target observed at 350 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

This western blot image is a comparison between ab175933 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

All lanes : Anti-53BP1 antibody [EPR2172(2)] (ab175933) at 1/1000 dilution (purified)

Lane 1 : Mouse heart tissue lysate
Lane 2 : Mouse brain tissue lysate

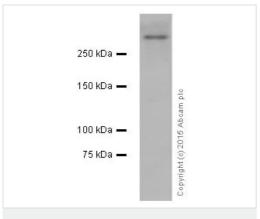
Lysates/proteins at 10 µg per lane.

Secondary

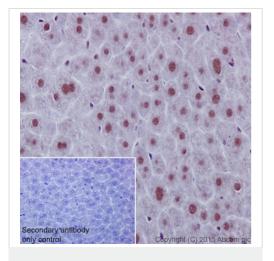
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 214 kDa **Observed band size:** 450 kDa

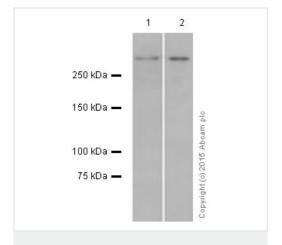
Blocking and dilution buffer: 5% NFDM /TBST.



Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-53BP1 antibody
[EPR2172(2)] (ab175933)



Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

Anti-53BP1 antibody [EPR2172(2)] (ab175933) at 20 µg (purified) + Rat heart tissue lysate at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 214 kDa **Observed band size:** 450 kDa

Blocking and dilution buffer: 5% NFDM /TBST.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue labelling 53BP1 with purified ab175933 at a dilution of 1/60. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

All lanes : Anti-53BP1 antibody [EPR2172(2)] (ab175933) at 1/5000 dilution (purified)

Lane 1: Human fetal heart tissue lysate

Lane 2: HeLa whole cell lysate

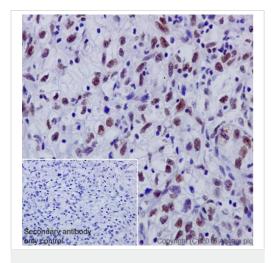
Lysates/proteins at 20 µg per lane.

Secondary

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

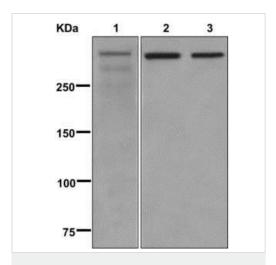
Predicted band size: 214 kDa **Observed band size:** 450 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-53BP1 antibody
[EPR2172(2)] (ab175933)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver carcinoma tissue labelling 53BP1 with purified ab175933 at a dilution of 1/60. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

All lanes : Anti-53BP1 antibody [EPR2172(2)] (ab175933) at 1/1000 dilution (unpurified)

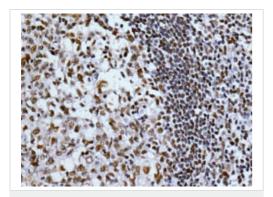
Lane 1: HepG2 cell lysate

Lane 2: Human fetal brain lysate

Lane 3: Human fetal heart lysate

Lysates/proteins at 1/10 dilution per lane.

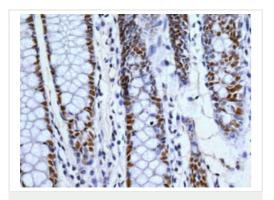
Predicted band size: 214 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-53BP1 antibody
[EPR2172(2)] (ab175933)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling 53BP1 with unpurified ab175933 at a dilution of 1/100.

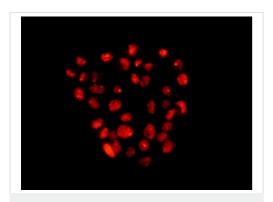
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-53BP1 antibody
[EPR2172(2)] (ab175933)

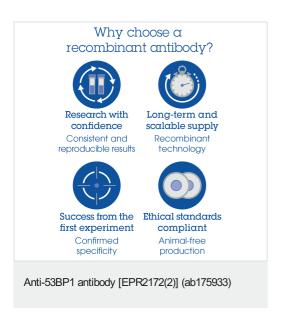
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling 53BP1 with unpurified ab175933 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling 53BP1 with unpurified ab175933 at a dilution of 1/100.



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