abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

★★★★★ 2 Abreviews 30 References 15 Images

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 within Human 53BP1 aa 1-100 (Cysteine residue). The exact sequence is proprietary. Database link: **Q12888**
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**. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.**

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	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab175933 in the following tested applications.

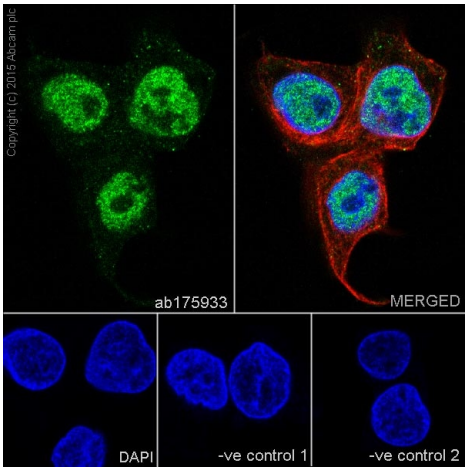
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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 disease	Note=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. Hyper-phosphorylated 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. Methylation of histone H4 at 'Lys-20' is required for efficient localization to double strand breaks.

Images

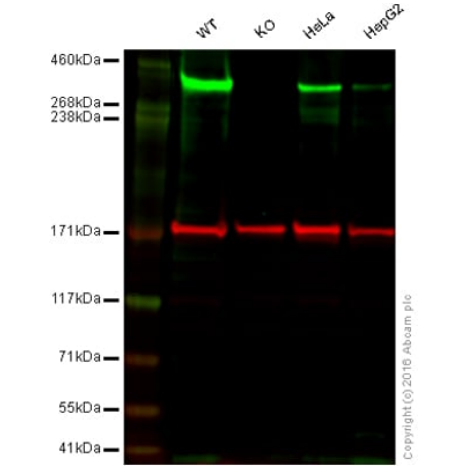


Immunocytochemistry/ Immunofluorescence - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

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 IgG (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 IgG (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).

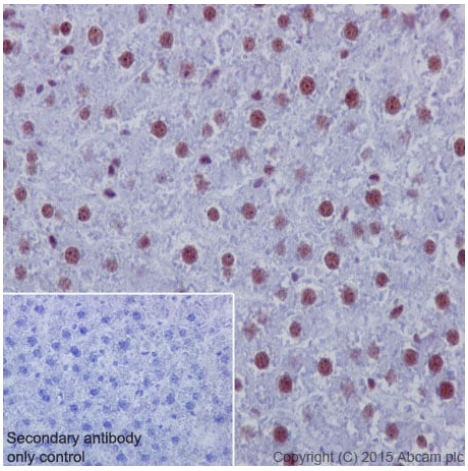
Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



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

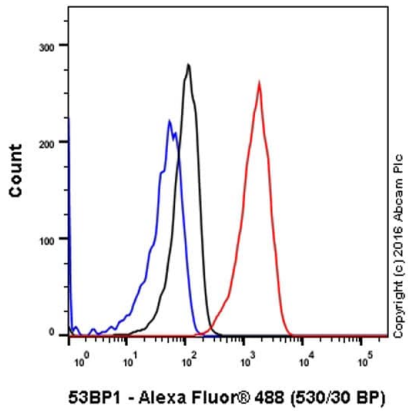
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: 53BP1 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (40 µg)
Lane 4: HepG2 cell lysate (40 µg)

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 **ab18058** (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 (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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 IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

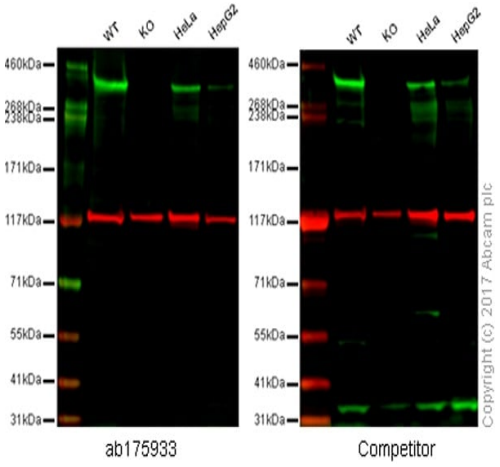


Flow Cytometry (Intracellular) - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

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 IgG (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)



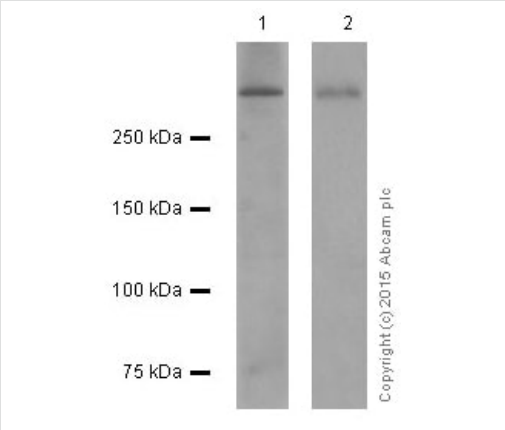
Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

Lane 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 - 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)

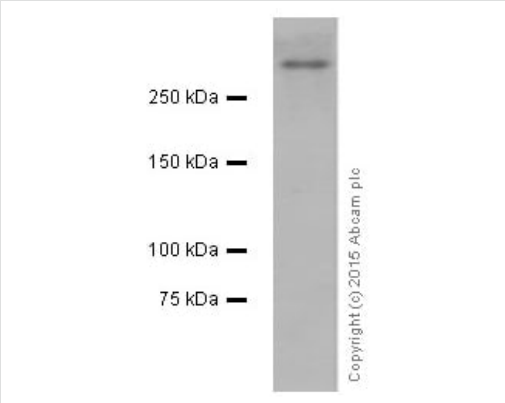
Lane 1 : Mouse heart tissue lysate
Lane 2 : Mouse brain tissue lysate

Lysates/proteins at 10 µg per lane.

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

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

Blocking and dilution buffer: 5% NFDM /TBST.



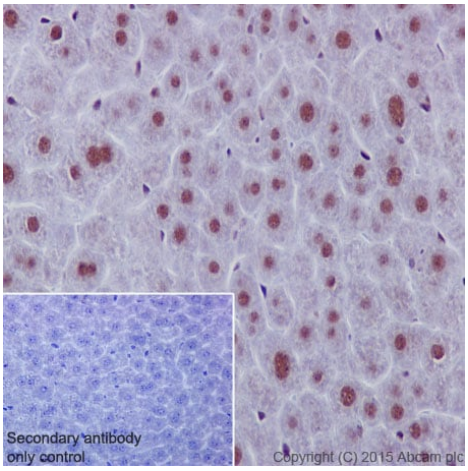
Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

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

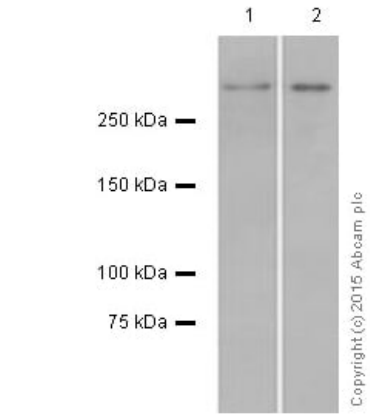
Predicted band size: 214 kDa
Observed band size: 450 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



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

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.



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

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

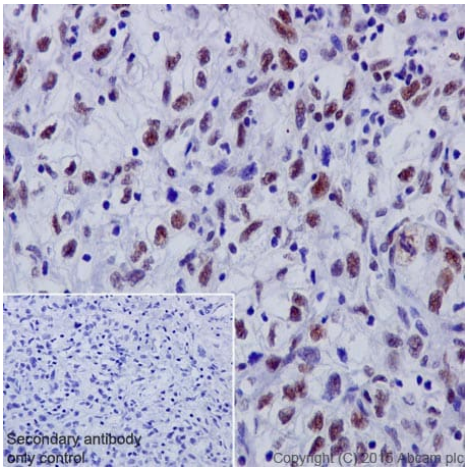
Lane 1 : Human fetal heart tissue lysate
Lane 2 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

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

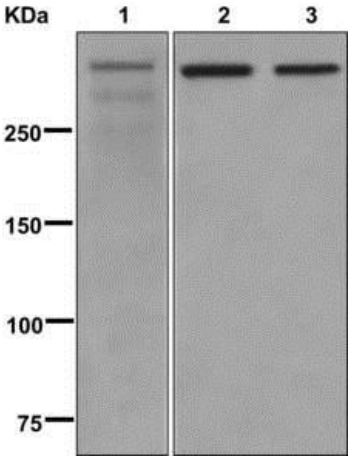
Predicted band size: 214 kDa
Observed band size: 450 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - 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 IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



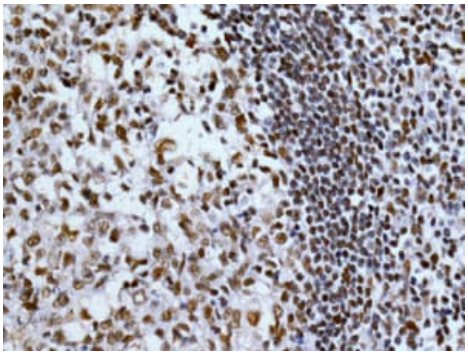
Western blot - Anti-53BP1 antibody [EPR2172(2)] (ab175933)

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.

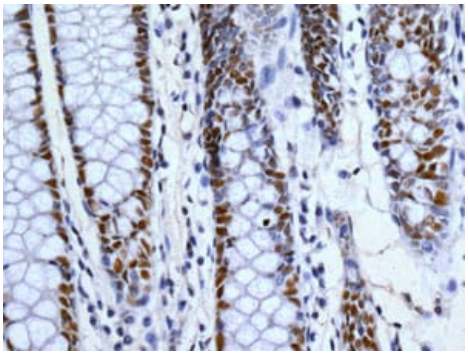
Predicted band size: 214 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.

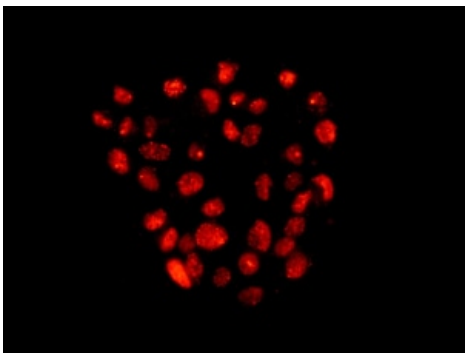
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

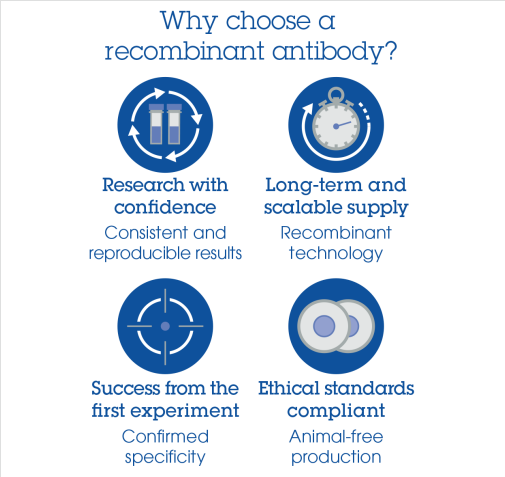
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling 53BP1 with unpurified ab175933 at a dilution of 1/100.

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.



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

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