abcam

Product datasheet

Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free ab249845

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR2173(2)] to 53BP1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P **Unsuitable for:** Flow Cyt or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, and HepG2 lysates. Human fetal brain and heart lysates. IHC-P: Human cervical carcinoma and prostate hyperplasia tissue. ICC/IF: HepG2 cells.
General notes	ab249845 is the carrier-free version of **ab175188**. Our **carrier-free** 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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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
Purity	Affinity purified
Clonality	Monoclonal
Clone number	EPR2173(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab249845 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
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 214 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

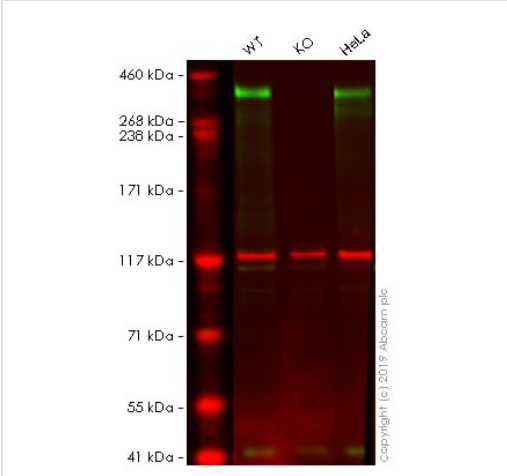
Application notes Is unsuitable for Flow Cyt or IP.

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



Western blot - Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free (ab249845)

All lanes : Anti-53BP1 antibody [EPR2173(2)] (**ab175188**) at 1/50000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : TP53BP1 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate

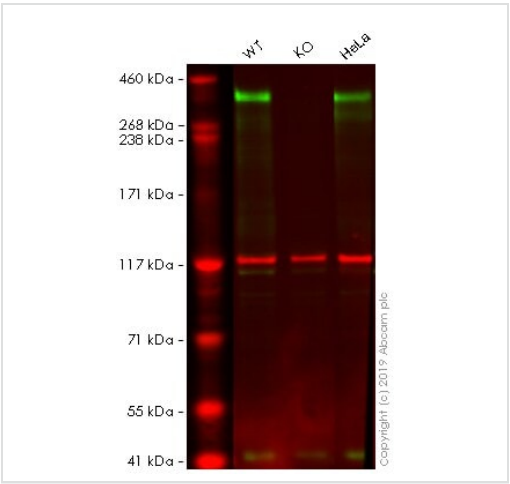
Lysates/proteins at 20 µg per lane.

Predicted band size: 214 kDa

This data was developed using **ab175188**, the same antibody clone in a different buffer formulation.

Lanes 1 - 3: Merged signal (red and green). Green - **ab175188** observed at 213 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

ab175188 was shown to recognize 53BP1 in wild-type HAP1 cells as signal was lost at the expected MW in TP53BP1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and TP53BP1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. **ab175188** and **ab130007** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/50000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



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All lanes : Anti-53BP1 antibody [EPR2173(2)] (**ab175188**) at 1/50000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : TP53BP1 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate

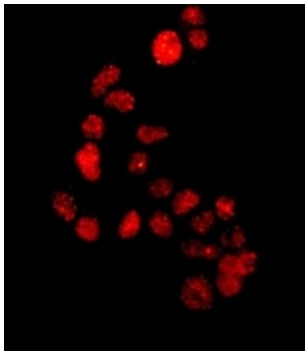
Lysates/proteins at 20 µg per lane.

Predicted band size: 214 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab175188**).

Lanes 1 - 3: Merged signal (red and green). Green - **ab175188** observed at 213 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

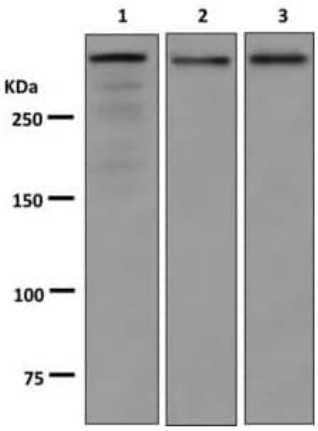
ab175188 was shown to recognize 53BP1 in wild-type HAP1 cells as signal was lost at the expected MW in TP53BP1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and TP53BP1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab175188 and **ab130007** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/50000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free (ab249845)

This data was developed using **ab175188**, the same antibody clone in a different buffer formulation.

Immunofluorescence analysis of HepG2 cells labeling 53BP1 with **ab175188** at a 1/100 dilution.



Western blot - Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free (ab249845)

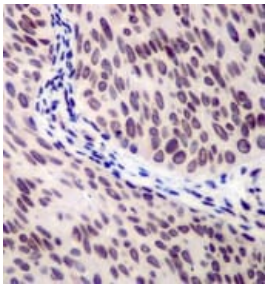
All lanes : Anti-53BP1 antibody [EPR2173(2)] (**ab175188**) at 1/50000 dilution

Lane 1 : Human fetal heart tissue lysate
Lane 2 : HepG2 cell lysate
Lane 3 : Human fetal brain tissue lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 214 kDa

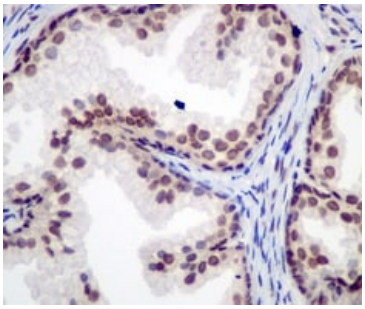
This data was developed using **ab175188**, the same antibody clone in a different buffer formulation.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free (ab249845)

This data was developed using **ab175188**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human cervical carcinoma tissue labeling 53BP1 with **ab175188** at a 1/50 dilution. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free (ab249845)

This data was developed using **ab175188**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human prostate hyperplasia tissue labeling 53BP1 with **ab175188** at a 1/50 dilution. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Anti-53BP1 antibody [EPR2173(2)] - BSA and Azide free (ab249845)

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