abcam

Product datasheet

Anti-PARP1 antibody [E102] ab32138





★★★★ 6 Abreviews 116 References 8 Images

Overview

Product name Anti-PARP1 antibody [E102]

Description Rabbit monoclonal [E102] to PARP1

Host species Rabbit

Specificity This antibody recognises both pro-form and p25 cleaved form of PARP1.

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

Unsuitable for: IP

Reacts with: Human Species reactivity

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

Positive control WB: Wild type HAP1 whole cell lysate; HeLa whole cell lysate (ab150035); HEK-293T cell lysate;

IHC-P: Human breast carcinoma tissue; ICC/IF: HeLa cells; Flow Cyt (intra): HeLa cells, HAP1

cells. WB: Jurkat whole cell lysate.

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**.

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

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long Storage instructions

term. Avoid freeze / thaw cycle.

Storage buffer

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number E102 Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32138 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	★★★★☆ (4)	1/1000 - 1/10000. Predicted molecular weight: 113 kDa. Existing as a 113 kDa nuclear protein, PARP1 is cleaved between amino acids Asp214 and Gly215 to yield two fragments of 29 kDa (N-terminal catalytic domain) and 85 kDa (C-terminal DNA-binding domain)
IHC-P	★★★★ (1)	1/200. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/25.
Flow Cyt (Intra)		1/20 - 1/50. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★ (1)	1/100.

Application notes

Is unsuitable for IP.

Target

Function Involved in the base excision repair (BER) pathway, by catalyzing the poly(ADP-ribosyl)ation of a limited number of acceptor proteins involved in chromatin architecture and in DNA metabolism.

This modification follows DNA damages and appears as an obligatory step in a

detection/signaling pathway leading to the reparation of DNA strand breaks. Mediates the poly(ADP-ribosyl)ation of APLF and CHFR. Positively regulates the transcription of MTUS1 and negatively regulates the transcription of MTUS2/TIP150.

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Sequence similarities Contains 1 BRCT domain.

Contains 1 PARP alpha-helical domain. Contains 1 PARP catalytic domain. Contains 2 PARP-type zinc fingers.

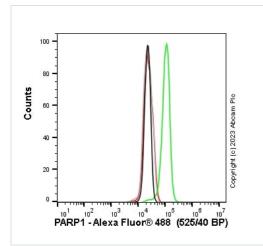
Post-translational modifications

Phosphorylated by PRKDC. Phosphorylated upon DNA damage, probably by ATM or ATR. Poly-ADP-ribosylated by PARP2. Poly-ADP-ribosylation mediates the recruitment of CHD1L to

DNA damage sites.

S-nitrosylated, leading to inhibit transcription regulation activity.

Images

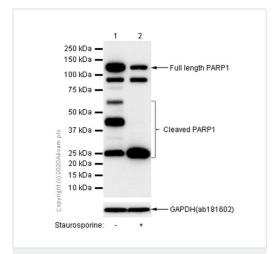


Flow Cytometry (Intracellular) - Anti-PARP1 antibody [E102] (ab32138)

Flow cytometry overlay histogram showing wild-type Hap1 (green line) and PARP1 knockout Hap1 stained with ab32138 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab32138) (1x 10^6 in 100μ l at $0.04~\mu$ g/ml (1/55750)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, PARP1 knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-PARP1 antibody [E102] (ab32138)

All lanes : Anti-PARP1 antibody [E102] (ab32138) at 1/1000 dilution (Purified)

Lane 1 : Untreated Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

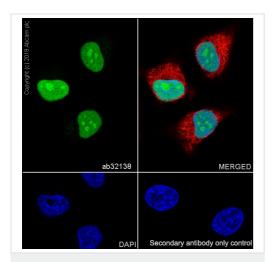
Lane 2: Jurkat (Human T cell leukemia T lymphocyte) treated with 1µM staurosporine for 4 hours whole cell lysate

Secondary

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

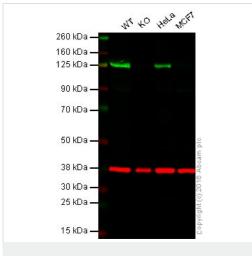
Predicted band size: 113 kDa

pro-form: 116kDa; p25 caspases cleaved form: 25kDa; proteolysis cleaved fragments: 58kDa and 42kDa



Immunocytochemistry/ Immunofluorescence - Anti-PARP1 antibody [E102] (ab32138)

Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PARP1 with purified ab32138 at 1/100 dilution (1.0 μ g/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% TritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 μ g/mL). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 μ g/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-PARP1 antibody [E102] (ab32138)

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

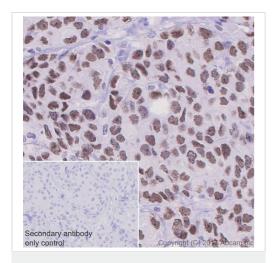
Lane 2: PARP1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: MCF7 whole cell lysate (20 µg)

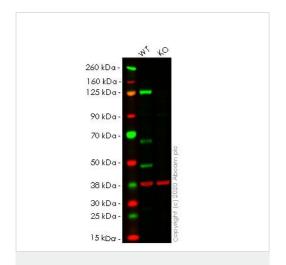
Lanes 1 - 4: Merged signal (red and green). Green - ab32138 observed at 125 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32138 was shown to specifically react with PARP1 when PARP1 knockout samples were used. Wild-type and PARP1 knockout samples were subjected to SDS-PAGE. ab32138 and <u>ab8245</u> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10 000 dilution respectively. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10 000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PARP1 antibody [E102] (ab32138)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling PARP1 with purified ab32138 at 1/200 dilution (0.51 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-PARP1 antibody [E102] (ab32138)

All lanes : Anti-PARP1 antibody [E102] (ab32138) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: PARP1 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

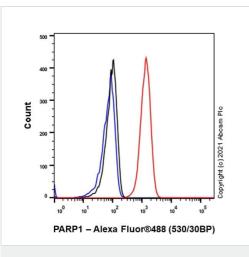
Performed under reducing conditions.

Predicted band size: 113 kDa **Observed band size:** 113 kDa

Lanes 1-2: Merged signal (red and green). Green - ab32138 observed at 113 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

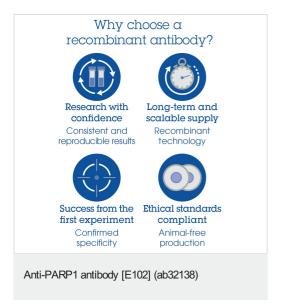
ab32138 was shown to react with PARP1 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266598 (knockout cell lysate ab257017) was used. Wild-type HEK-293T and PARP1 knockout HEK-293T cell lysates were subjected to SDS-PAGE. ab32138 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at

1 in 20000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-PARP1 antibody [E102] (ab32138)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling PARP1 with purified ab32138 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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