abcam

Product datasheet

Anti-Cleaved PARP1 antibody [E51] ab32064

KO VALIDATED Recombinant RabMAb

18 Abreviews 344 References 12 Images

Overview

Product name	Anti-Cleaved PARP1 antibody [E51]
Description	Rabbit monoclonal [E51] to Cleaved PARP1
Host species	Rabbit
Specificity	This antibody is specific for the p25 cleaved form of human PARP1.
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Chinese hamster
Immunogen	Synthetic peptide within Human Cleaved PARP1 aa 150-250. The exact sequence is proprietary. Database link: **P09874**
Positive control	WB: Jurkat whole cell lysate (**ab7899**). HeLa and RAW 264.7 whole cell lysate. HAP1, HeLa, NIH/3T3 and PC-12 treated with 1uM Staurosporine. Jukat cells treated with camptothecin. Jukat cells treated with 15-Acetoxyscirpenol. IHC-P: Rat colon tissue. Human ovarian cancer and breast carcinoma tissue.
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E51
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32064 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	★★★★☆ (7)	1/1000 - 1/10000. Predicted molecular weight: 25 kDa.
IHC-P	★★★★☆ (5)	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for ICC/IF.

Target

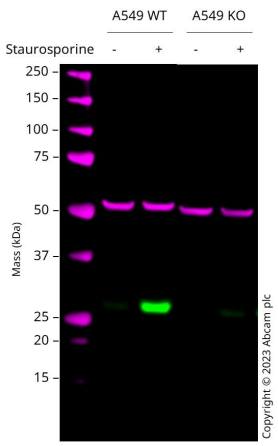
Function Involved in the base excision repair (BER) pathway, by catalyzing the poly(ADP-ribosyl)ation of a limited number of acceptor proteins involved in chromatin architecture and in DNA metabolism. This modification follows DNA damages and appears as an obligatory step in a detection/signaling pathway 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. With EEF1A1 and TXK, forms a complex that acts as a T-helper 1 (Th1) cell-specific transcription factor and binds the promoter of IFN-gamma to directly regulate its transcription, and is thus involved importantly in Th1 cytokine production. Required for PARP9 and DTX3L recruitment to DNA damage sites. PARP1-dependent PARP9-DTX3L-mediated ubiquitination promotes the rapid and specific recruitment of 53BP1/TP53BP1, UIMC1/RAP80, and BRCA1 to DNA damage sites.

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 and TXK.
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.

Cellular localization Nucleus. Nucleus, nucleolus. Localizes at sites of DNA damage.

Images



Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

All lanes : Anti-Cleaved PARP1 antibody [E51] (ab32064) at 1/10000 dilution

Lane 1 : Wild-type A549 control staurosporine (0 uM, 72 h) cell lysate
Lane 2 : Wild-type A549 treated staurosporine (3 uM, 24 h) cell lysate
Lane 3 : Wild-type A549 control staurosporine (3 uM, 72 h) cell lysate
Lane 4 : PARP1 knockout A549 treated staurosporine (3 uM, 24 h) cell lysate

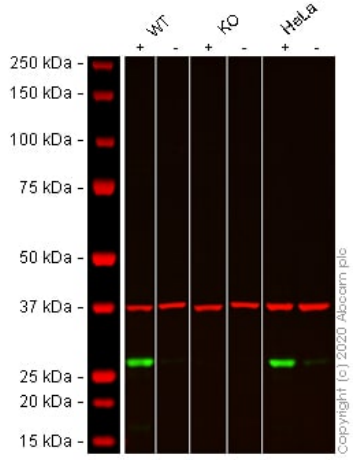
Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 27 kDa

Western blot: Anti-PARP1 antibody [E51] (ab32064) staining at 1/10000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab32064 was shown to bind specifically to PARP1. A band was observed at 27 kDa in wild-type A549 cell lysates with no signal observed at this size in PARP1 knockout cell line. To generate this image, wild-type and PARP1 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

All lanes : Anti-Cleaved PARP1 antibody [E51] (ab32064) at 1/10000 dilution

Lane 1 : Wild-type (1uM Staurosporine for 3hrs) HAP1 cell lysate
Lane 2 : Wild-type (Staurosporine control) HAP1 cell lysate
Lane 3 : PARP1 knockout (1uM Staurosporine for 3hrs) HAP1 cell lysate
Lane 4 : PARP1 knockout (Staurosporine control) HAP1 cell lysate
Lane 5 : HeLa (1uM Staurosporine for 3hrs) cell lysate
Lane 6 : HeLa cell lysate

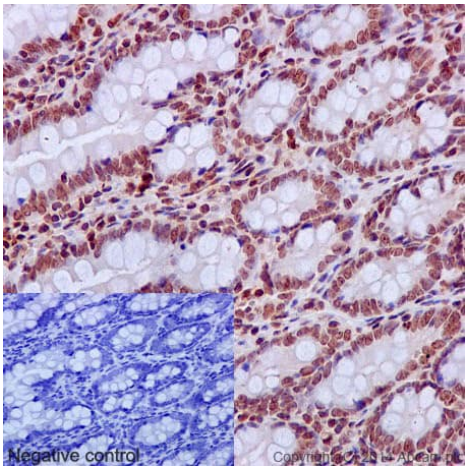
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 27 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab32064 observed at 27 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

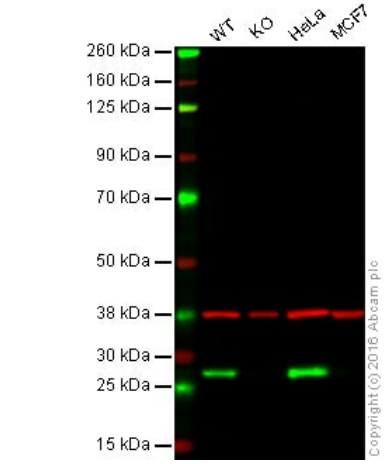
ab32064 was shown to react with Cleaved PARP1 in wild-type HAP1 cells in Western blot with loss of signal observed in PARP1 knockout sample.Wild-type HAP1 and PARP1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab32064 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 h at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cleaved PARP1 antibody [E51] (ab32064)

Immunohistochemical staining of paraffin embedded rat colon with purified ab32064 at a working dilution of 1/100. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counterstained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control (inset).



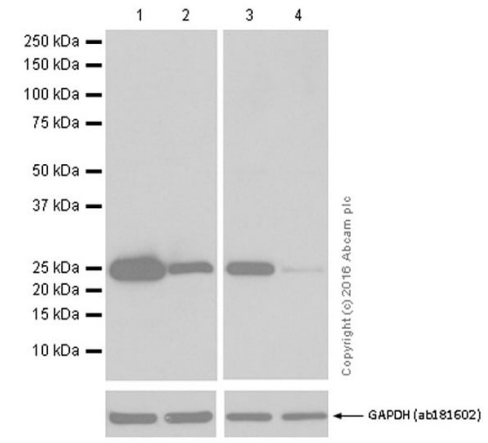
Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

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 - ab32064 observed at 30 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32064 was shown to specifically react with PARP1 when PARP1 knockout samples were used. Wild-type and PARP1 knockout samples were subjected to SDS-PAGE. Ab32064 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilutions.

Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) **ab216773** and 680CW Goat anti Mouse secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

All lanes : Anti-Cleaved PARP1 antibody [E51] (ab32064) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysates treated with 1uM Staurosporine for 3 hours
Lane 2 : Untreated HeLa whole cell lysates
Lane 3 : NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysates treated with 1uM Staurosporine for 3 hours
Lane 4 : Untreated NIH/3T3 whole cell lysates

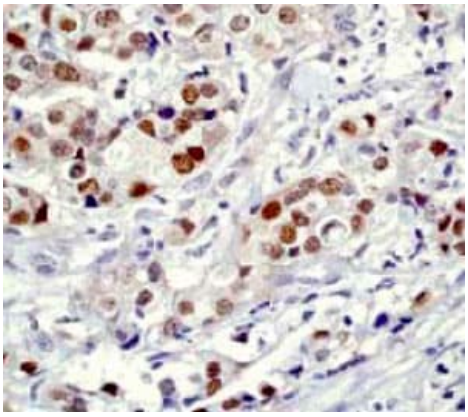
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 25 kDa
Observed band size: 25 kDa

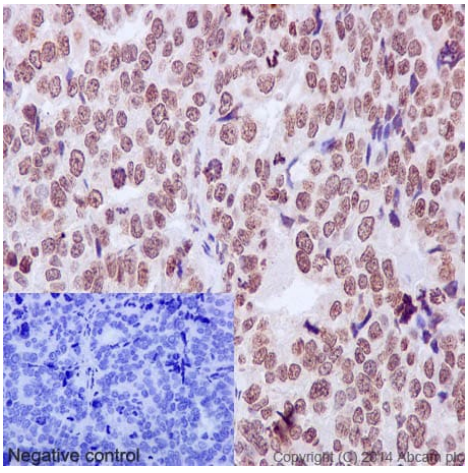
Blocking/Dilution buffer 5% NFDM/TBST

Exposure time :
Lane 1,2: 1 second
Lane 3,4: 8 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cleaved PARP1 antibody [E51] (ab32064)

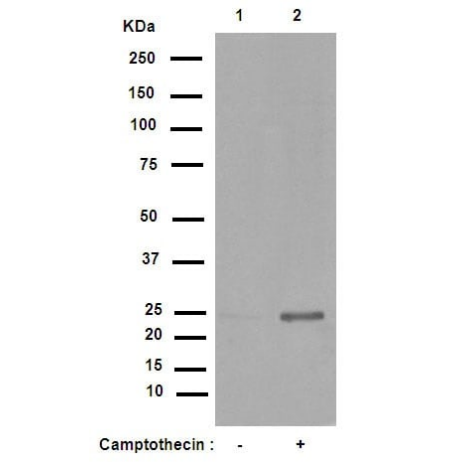
Immunohistochemical staining of paraffin embedded human breast carcinoma tissue with unpurified ab32064 at a 1/100 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cleaved PARP1 antibody [E51] (ab32064)

Immunohistochemical staining of paraffin embedded human ovarian carcinoma with purified ab32064 at a working dilution of 1 in 100. The secondary antibody used is a HRP polymer for rabbit IgG. Counterstained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control (inset).



Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

All lanes : Anti-Cleaved PARP1 antibody [E51] (ab32064) at 1/10000 dilution

Lane 1 : Untreated Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate
Lane 2 : Jurkat cell lysate treated with camptothecin

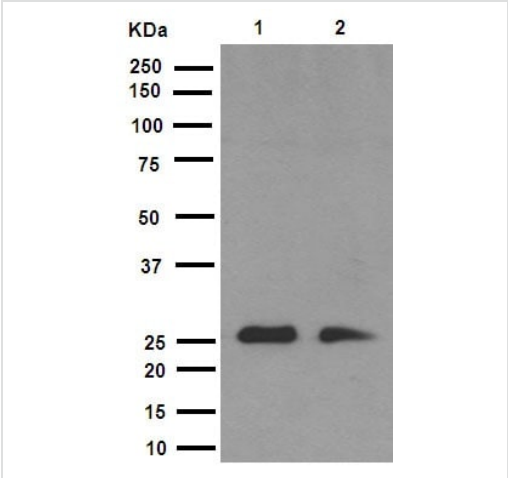
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 25 kDa
Observed band size: 25 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

All lanes : Anti-Cleaved PARP1 antibody [E51] (ab32064) at 1/1000 dilution

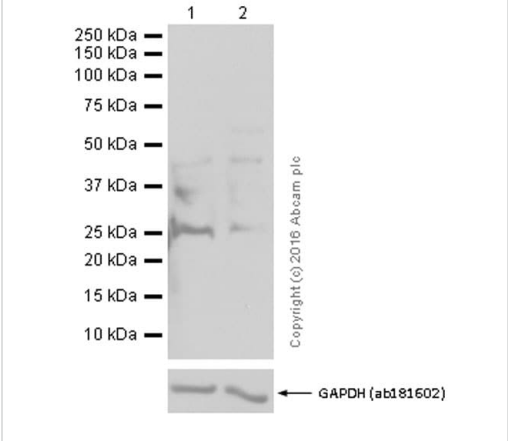
Lane 1 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate
Lane 2 : NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 25 kDa
Observed band size: 25 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

All lanes : Anti-Cleaved PARP1 antibody [E51] (ab32064) at 1/1000 dilution

Lane 1 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysates treated with 1uM Staurosporine for 3 hours
Lane 2 : Untreated PC-12 whole cell lysates

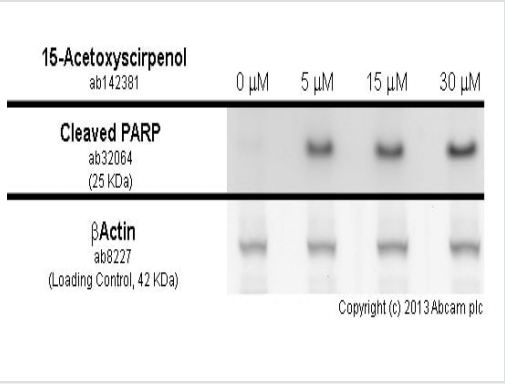
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 30 seconds

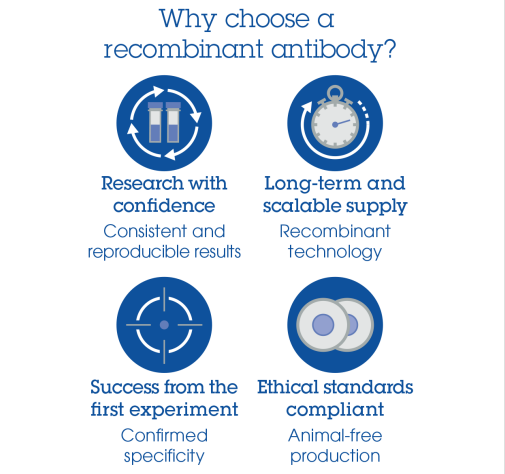
Blockinng/Diluting buffer 5% NFDM/TBST



Western blot - Anti-Cleaved PARP1 antibody [E51] (ab32064)

Jurkat (Human T cell leukemia cell line from peripheral blood) cells were incubated at 37°C for 24 hours with vehicle control (0 µM) and different concentrations of 15-Acetoxyscirpenol (**ab142381**). Increased expression of cleaved PARP1 (ab32064) in Jurkat cells correlates with an increase in 15-Acetoxyscirpenol concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab32064 at 1/10000 dilution and **ab8227** at 1 µg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (**ab97051**) at 1/10000 and visualised using ECL development solution.



Anti-Cleaved PARP1 antibody [E51] (ab32064)

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