Anti-Cleaved PARP Antibody [SU0314] - BSA and Azide free

HA750136

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IP
Molecular Wt:	Predicted band size: 89 kDa
Clone number:	SU0314

Description: Poly (ADP-ribose) polymerase (PARP) is a family of proteins involved in a number of cellular processes such as DNA repair, genomic stability, and programmed cell death. The main role of PARP (found in the cell nucleus) is to detect and initiate an immediate cellular response to metabolic, chemical, or radiation-induced single-strand DNA breaks (SSB) by signaling the enzymatic machinery involved in the SSB repair. Once PARP detects a SSB, it binds to the DNA, undergoes a structural change, and begins the synthesis of a polymeric adenosine diphosphate ribose (poly (ADP-ribose) or PAR) chain, which acts as a signal for the other DNA-repairing enzymes. Target enzymes include DNA ligase III (LigIII), DNA polymerase beta (polβ), and scaffolding proteins such as X-ray cross-complementing gene 1 (XRCC1). After repairing, the PAR chains are degraded via Poly(ADP-ribose) glycohydrolase (PARG). PARP enzymes are essential in a number of cellular functions, including expression of inflammatory genes: PARP1 is required for the induction of ICAM-1 gene expression by cardiac myocytes and smooth muscle cells, in response to TNF.

Immunogen: Synthetic peptide within Human PARP1 aa 200-249 / 1,014.

Positive control: Jurkat cell lysate, A549 cell lysate, Hela, Daudi cell lysates.

Subcellular location: Nucleus.

Database links: SwissProt: P09874 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100-1:250
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

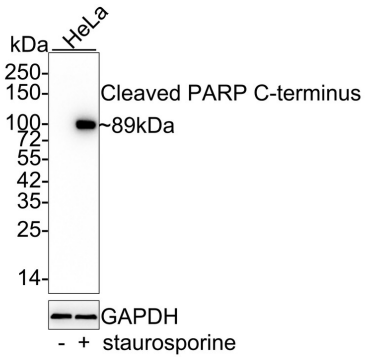

Fig1: Western blot analysis of Cleaved PARP on different lysates with Rabbit anti-Cleaved PARP antibody (HA750136) at 1/2,000 dilution.

Lane 1: HeLa whole cell lysate
Lane 2: HeLa treated with 1μM staurosporine for 3 hours whole cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 89 kDa
Observed band size: 89 kDa

Exposure time: 1 minute 9 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750136) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

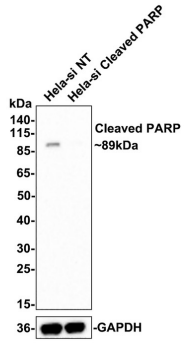

Fig2: Western blot analysis of Cleaved PARP on different lysates with Rabbit anti-Cleaved PARP antibody (HA750136) at 1/500 dilution.

Lane 1: Hela-si NT cell lysate (10 μg/Lane)
Lane 2: Hela-si Cleaved PARP cell lysate (10 μg/Lane)

Predicted band size: 89 kDa
Observed band size: 89 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

ET1608-10 was shown to specifically react with Cleaved PARP in Hela-si NT cells. No band was observed when Hela-si Cleaved PARP sample was tested. Hela-si NT and Hela-si Cleaved PARP samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1608-10, 1/500) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

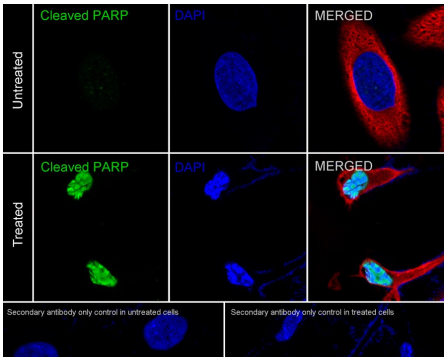

Fig3: Immunocytochemistry analysis of HeLa cells treated with 1μM staurosporine for 180 minutes labeling Cleaved PARP with Rabbit anti-Cleaved PARP antibody (HA750136) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Cleaved PARP antibody (HA750136) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

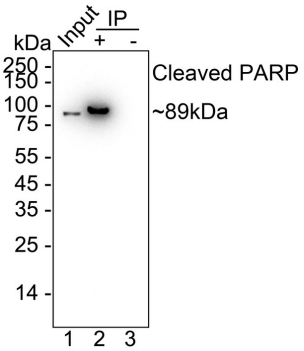

Fig4: Cleaved PARP was immunoprecipitated from 0.2 mg Daudi cell lysate with HA750136 at 2 μg/10 μl beads. Western blot was performed from the immunoprecipitate using HA750136 at 1/500 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Daudi cell lysate (input)
Lane 2: HA750136 IP in Daudi cell lysate
Lane 3: Rabbit IgG instead of HA750136 in Daudi cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 12 seconds; ECL: K1801

Background References

1. Peh J et al. The Combination of Vemurafenib and Procaspase-3 Activation Is Synergistic in Mutant BRAF Melanomas. Mol Cancer Ther 15:1859-69 (2016).
2. Gao L et al. Glycyrrhizic acid alleviates bleomycin-induced pulmonary fibrosis in rats. Front Pharmacol 6:215 (2015).