

Anti-Cleaved PARP Antibody [SU0314]

ET1608-10



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 (pol β), 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	1-2 μ g/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

 华安生物
HUABIO
www.huabio.cn

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

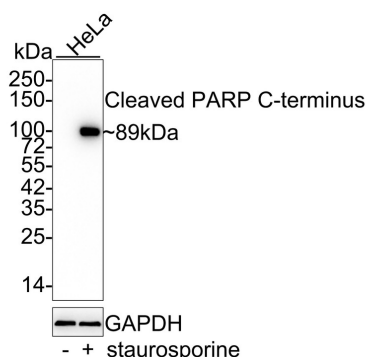


Fig1: Western blot analysis of Cleaved PARP on different lysates with Rabbit anti-Cleaved PARP antibody (ET1608-10) at 1/2,000 dilution.

Lane 1: HeLa whole cell lysate

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

Lysates/proteins at 20 μ g/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 (ET1608-10) at 1/2,000 dilution was used in 5% NFDM/TBST at 4 $^{\circ}$ C 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 (ET1608-10) at 1/500 dilution.

Lane 1: Hela-si NT cell lysate (10 μ g/Lane)

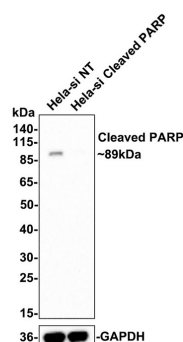
Lane 2: Hela-si Cleaved PARP cell lysate (10 μ g/Lane)

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.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUABIO
www.huabio.cn

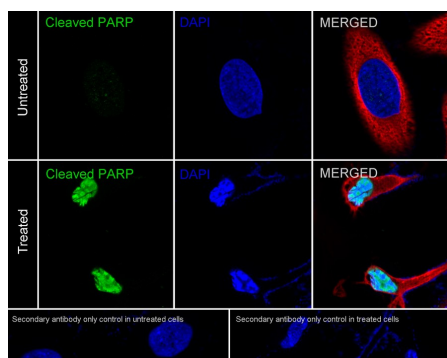


Fig3: Immunocytochemistry analysis of HeLa cells treated with 1 μ M staurosporine for 180 minutes labeling Cleaved PARP with Rabbit anti-Cleaved PARP antibody (ET1608-10) at 1/250 dilution.

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 (ET1608-10) at 1/250 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

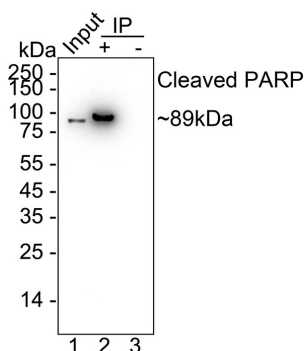


Fig4: Cleaved PARP was immunoprecipitated from 0.2 mg Daudi cell lysate with ET1608-10 at 2 μ g/10 μ l beads. Western blot was performed from the immunoprecipitate using ET1608-10 at 1/500 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Daudi cell lysate (input)

Lane 2: ET1608-10 IP in Daudi cell lysate

Lane 3: Rabbit IgG instead of ET1608-10 in Daudi cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 12 seconds; ECL: K1801

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Peh J et al. The Combination of Vemurafenib and Procaspace-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).

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物
HUABIO
www.huabio.cn

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation