Apoptosis Antibody Sampler Kit

K2001

Contains Product	Specification	Applications	Species reactivity	MW(kDa)
Caspase-3[ET1602-39]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H	32 kDa
Active Caspase-3[ET1602-47]	20μl	WB,IF-Cell,IF-Tissue,IHC-P	H	17 kDa
PARP[ET1608-56]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M	113 kDa
Cleaved PARP[ET1608-10]	20μl	WB,IF-Cell,IP,FC	H	89 kDa
Caspase-9[ET1610-95]	20μl	WB,IP	H,M	46/30/17/37 kDa
Active+Pro Caspase-9[R1308-12]	20μl	WB,IHC-P	H	46 kDa
pro Caspase-7[ET1612-28]	20μl	WB,IF-Cell,IF-Tissue,IHC-P	H	34 kDa
Cleaved-Caspase-7 p20[ER60002]	20μl	WB,IF,IHC-P	H,M,R	20 kDa
HRP-Alpaca anti-Rabbit IgG Fc, Recombinant VHH[HA1031]	100μl	IP,ELISA,IHC-P,WB	Rab	

Description: The Apoptosis Antibody Sampler Kit designed to provide you with a variety of trial-size antibodies in a convenient and cost-effective format. The kit can be used to detect the full length and cleaved products of Caspase-3, Caspase-7, Caspase-9, and PARP. And also includes secondary reagent for detection of these antibodies.

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

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

Background

Apoptosis is a regulated physiological process leading to cell death. Caspases, a family of cysteine acid proteases, are central regulators of apoptosis. Initiator caspases are closely coupled to proapoptotic signals.

Once activated, these caspases cleave and activate downstream effector caspases, which in turn cleave cytoskeletal and nuclear proteins like PARP, α-fodrin, DFF, and lamin A and induce apoptosis. Cytochrome c released from mitochondria is coupled to the activation of caspase-9, a key initiator caspase. Proapoptotic stimuli include FasL, TNF-α, DNA damage and ER stress. Fas and TNFR activate caspase-8 and -10, DNA damage leads to the activation of caspase-9 and ER stress leads to the calcium-mediated activation of caspase-12 (3). The inhibitor of apoptosis protein (IAP) family includes XIAP and survivin and functions by binding and inhibiting several caspases. Smac/Diablo, a mitochondrial protein, is released into the cytosol upon mitochondrial stress and competes with caspases for binding of IAPs. The interaction of Smac/Diablo with IAPs relieves the inhibitory effects of IAPs on caspases .

Database links: UniProt ID: P42574, P42574, P09874, P11103, P27008, P09874, P55211, Q8C3Q9, P55211, P55210, P55210

Images

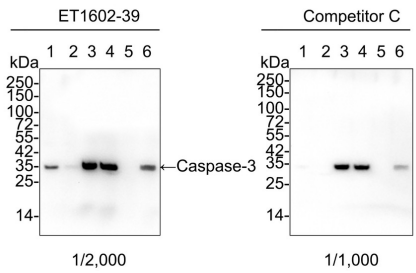

Fig1: Western blot analysis of Caspase-3 on different lysates with Rabbit anti-Caspase-3 antibody (ET1602-39) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 1μM staurosporine for 3 hours cell lysate
Lane 3: Jurkat cell lysate
Lane 4: Jurkat treated with 25μM Etoposide for 5 hours cell lysate
Lane 5: MCF7 cell lysate (negative)
Lane 6: HEK-293 cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 32 kDa
Observed band size: 32 kDa

Exposure time: 3 minutes 20 seconds;

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 (ET1602-39) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

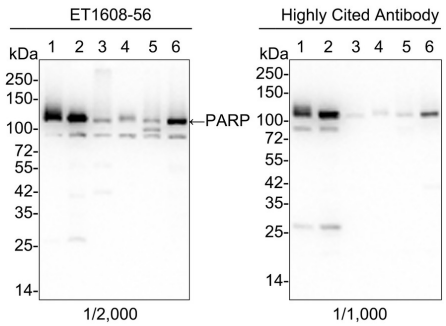

Fig2: Western blot analysis of PARP on different lysates with Rabbit anti-PARP antibody (ET1608-56) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: Jurkat cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 cell lysate (15 µg/Lane)
Lane 4: C2C12 cell lysate (15 µg/Lane)
Lane 5: C6 cell lysate (15 µg/Lane)
Lane 6: PC-12 cell lysate (15 µg/Lane)

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

Exposure time: 6 seconds;

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 (ET1608-56) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

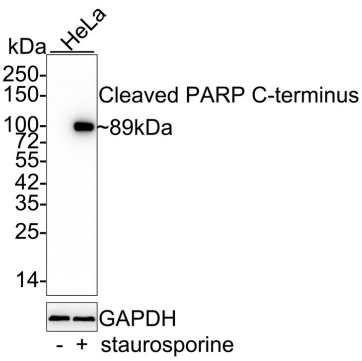

Fig3: Western blot analysis of Cleaved PARP on different lysates with Rabbit anti-Cleaved PARP antibody (ET1608-10) at 1/2,000 dilution.

Lane 1: HeLa whole cell lysate
Lane 2: HeLa treated with 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;

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℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

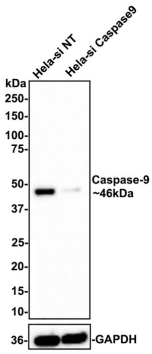

Fig4: Western blot analysis of Caspase-9 on different lysates with Rabbit anti-Caspase-9 antibody (ET1610-95) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si Caspase-9 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 46 kDa
Observed band size: 46 kDa

Exposure time: 2 minutes;

4-20% SDS-PAGE gel.

ET1610-95 was shown to specifically react with Caspase-9 in Hela-si NT cells. Weakened band was observed when Hela-si Caspase-9 sample was tested. Hela-si NT and Hela-si Caspase-9 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 (ET1610-95, 1/1,000) 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 1:100,000 dilution was used for 1 hour at room temperature.

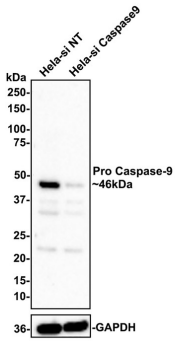

Fig5: Western blot analysis of Active+Pro Caspase-9 on different lysates with Rabbit anti-Active+Pro Caspase-9 antibody (R1308-12) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si Caspase-9 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 46 kDa
Observed band size: 46 kDa

Exposure time: 5 minutes;

4-20% SDS-PAGE gel.

R1308-12 was shown to specifically react with Active+Pro Caspase-9 in Hela-si NT cells. Weakened band was observed when Hela-si Active+Pro Caspase-9 sample was tested. Hela-si NT and Hela-si Active+Pro Caspase-9 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 (R1308-12, 1/1,000) 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 1:100,000 dilution was used for 1 hour at room temperature.

Background References

1. Baker, S.J. and Reddy, E.P. (1998) Oncogene 17, 3261-3270.
2. Budihardjo, I. et al. (1999) Annu. Rev. Cell Dev. Biol. 15, 269-290.
3. Nakagawa, T. et al. (2000) Nature 403, 98-103.
4. Deveraux, Q. L. et al. (1998) EMBO J. 17, 2215-2223.
5. Li, F. et al. (1998) Nature 396, 580-584.
6. Du, C. et al. (2000) Cell 102, 33-42.