Anti-Active+Pro Caspase-9 Antibody [JJ08-05] ET1701-22

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 46 kDa
Clone number:	JJ08-05

Description: Caspase-9 is an enzyme that in humans is encoded by the CASP9 gene. It is an initiator caspase, critical to the apoptotic pathway found in many tissues. Caspase-9 belongs to a family of caspases, cysteine-aspartic proteases involved in apoptosis and cytokine signalling. Apoptotic signals cause the release of cytochrome c from mitochondria and activation of apaf-1 (apoptosome), which then cleaves the pro-enzyme of caspase-9 into the active dimer form. Correct caspase-9 function is required for apoptosis, leading to the normal development of the central nervous system. Caspase-9 has multiple additional cellular functions that are independent of its role in apoptosis. Nonapoptotic roles of caspase-9 include regulation of necroptosis, cellular differentiation, innate immune response, sensory neuron maturation, mitochondrial homeostasis, corticospinal circuit organization, and ischemic vascular injury. Without correct function, abnormal tissue development can occur leading to abnormal function, diseases and premature death. Caspase-9 loss-of-function mutations have been associated with immunodeficiency/lymphoproliferation, neural tube defects, and Li-Fraumeni-like syndrome. Increased caspase-9 activity is implicated in the progression of amyotrophic lateral sclerosis, retinal detachment, and slow-channel syndrome, as well as various other neurological, autoimmune, and cardiovascular disorders.

Immunogen: Synthetic peptide within Human Caspase-9 aa 20-69 / 416.

Positive control: HeLa cell lysate, HeLa treated with 1μM staurosporine for 4 hours cell lysate, HeLa treated with 3μM staurosporine for 4 hours cell lysate, human placenta tissue, human skin tissue, human cervix uteri tissue, Jurkat.

Subcellular location: Cytosol,Nucleus.

Database links: SwissProt: P55211 Human

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:50-1:200
FC	1:10-1:50

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

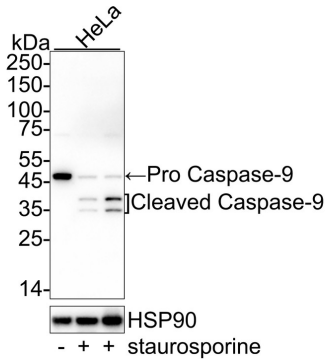

Fig1: Western blot analysis of Active+Pro Caspase-9 on different lysates with Rabbit anti-Active+Pro Caspase-9 antibody (ET1701-22) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 1μM staurosporine for 4 hours cell lysate
Lane 3: HeLa treated with 3μM staurosporine for 4 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 46 kDa
Observed band size: 46/37/35 kDa

Exposure time: 3 minutes; 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 (ET1701-22) at 1/1,000 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.

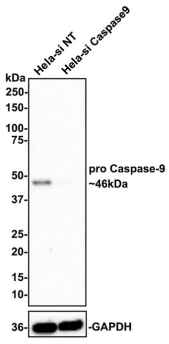

Fig2: Western blot analysis of pro Caspase-9 on different lysates with Rabbit anti-pro Caspase-9 antibody (ET1701-22) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate (10 μg/Lane)
Lane 2: Hela-si Caspase-9 cell lysate (10 μg/Lane)

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

Exposure time: 5 minutes;
4-20% SDS-PAGE gel.

ET1701-22 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 (ET1701-22, 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.

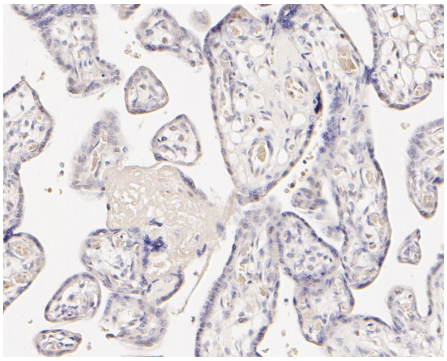

Fig3: Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-Active+Pro Caspase-9 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-22, 1/100) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

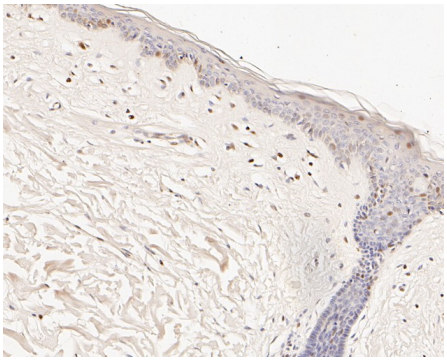

Fig4: Immunohistochemical analysis of paraffin-embedded human skin tissue using anti-Active+Pro Caspase-9 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-22, 1/100) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

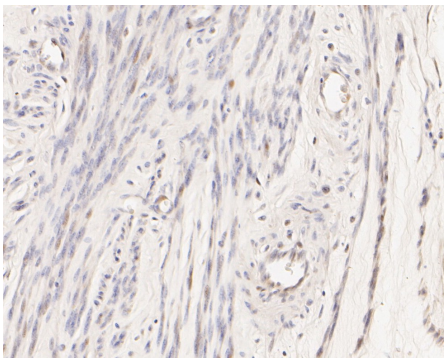

Fig5: Immunohistochemical analysis of paraffin-embedded human cervix uteri tissue using anti-Active+Pro Caspase-9 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-22, 1/100) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

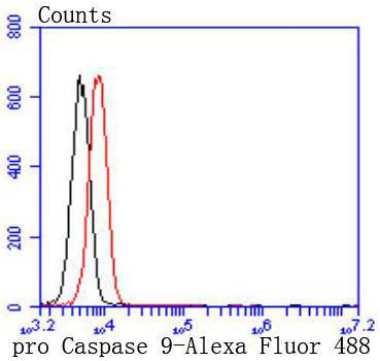

Fig6: Flow cytometric analysis of Jurkat cells with pro Caspase 9 antibody at 1/50 dilution (red) compared with an unlabelled control (cells without incubation with primary antibody; black). Alexa Fluor 488-conjugated goat anti rabbit IgG was used as the secondary antibody

Background References

1. Arango-Gonzalez B et al. Identification of a common non-apoptotic cell death mechanism in hereditary retinal degeneration. PLoS One 9:e112142 (2014).
2. Catuogno S et al. miR-34c may protect lung cancer cells from paclitaxel-induced apoptosis. Oncogene : (2012).