Anti-Caspase-3 Antibody [SR03-01]

ET1602-39

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 32 kDa
Clone number:	SR03-01

Description: Caspase-3 is a caspase protein that interacts with caspase-8 and caspase-9. It is encoded by the CASP3 gene. CASP3 orthologs have been identified in numerous mammals for which complete genome data are available. Unique orthologs are also present in birds, lizards, lissamphibians, and teleosts. Caspase-3 shares many of the typical characteristics common to all currently-known caspases. For example, its active site contains a cysteine residue (Cys-163) and histidine residue (His-121) that stabilize the peptide bond cleavage of a protein sequence to the carboxy-terminal side of an aspartic acid when it is part of a particular 4-amino acid sequence. This specificity allows caspases to be incredibly selective, with a 20,000-fold preference for aspartic acid over glutamic acid. A key feature of caspases in the cell is that they are present as zymogens, termed procaspases, which are inactive until a biochemical change causes their activation. Each procaspase has an N-terminal large subunit of about 20 kDa followed by a smaller subunit of about 10 kDa, called p20 and p10, respectively.

Immunogen: Synthetic peptide within human Caspase-3 aa 60-100.

Positive control: HeLa cell lysate, Jurkat cell lysate, HEK-293 cell lysate, Hela, human tonsil tissue, human spleen tissue.

Subcellular location: Cytoplasm

Database links: SwissProt P42574 Human

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:50
IF-Tissue	1:50
IHC-P	1:50
FC	1:20-1:50
IP	Use at an assay dependent concentration.

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

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

Purity: Protein A affinity purified.

Images

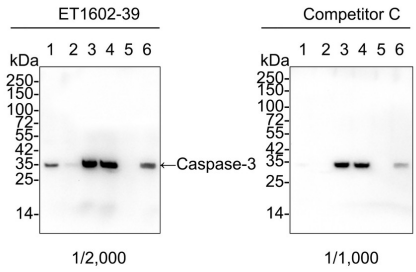

Fig1: Western blot analysis of Caspase-3 on different lysates with Rabbit anti-Caspase-3 antibody (ET1602-39) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

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.

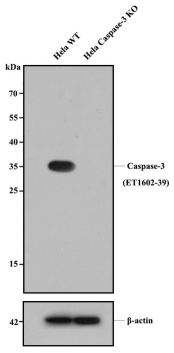

Fig2: All lanes: Western blot analysis of Caspase-3 with anti-Caspase-3 antibody [SR03-01] (ET1602-39) at 1:500 dilution.
Lane 1: Wild-type Hela whole cell lysate (20 μg).
Lane 2: Caspase-3 knockout Hela whole cell lysate (20 μg).

ET1602-39 was shown to specifically react with Caspase-3 in wild-type Hela cells. No band was observed when Caspase-3 knockout sample was tested. Wild-type and Caspase-3 knockout 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 (ET1602-39, 1/500) and Loading control antibody (Rabbit anti-β-actin, R1207-1, 1/1,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

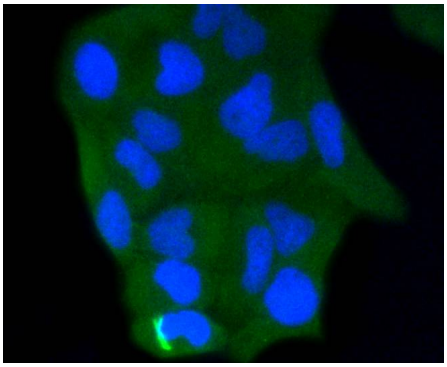

Fig3: ICC staining of Caspase-3 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1602-39, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

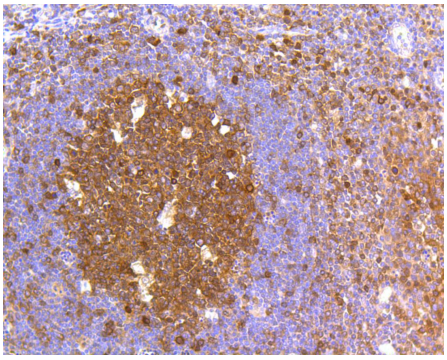

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Caspase-3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-39, 1/50) 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.

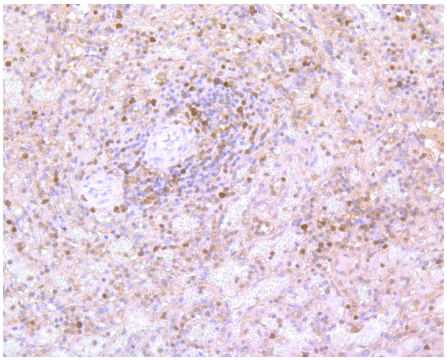

Fig5: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-Caspase-3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1602-39, 1/50) 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.

Background References

1. Li H et al. Protective effect of ginsenoside Rg1 on lidocaine-induced apoptosis. Mol Med Rep 9:395-400 (2014).
2. Cejkova J et al. Suppression of alkali-induced oxidative injury in the cornea by mesenchymal stem cells growing on nanofiber scaffolds and transferred onto the damaged corneal surface. Exp Eye Res 116:312-23 (2013).