Anti-Caspase-9 Antibody [SZ29-01]

ET1603-27

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 46 kDa
Clone number:	SZ29-01

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 homologs have been identified in all mammals for which they are known to exist, such as Mus musculus and Pan troglodytes. 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. Regulation of this enzyme occurs through phosphorylation by an allosteric inhibitor, inhibiting dimerization and inducing a conformational change. 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.

Immunogen:	Synthetic peptide within Human Caspase-9 aa 289-338 / 416.
Positive control:	C2C12 cell lysate, Hela cell lysate, NIH/3T3, HepG2 cell, A549 cell, human tonsil tissue, human cervix carcinoma tissue, human colon tissue, human colon carcinoma tissue, mouse spleen tissue.
Subcellular location:	Mitochondrion, cytoplasm, cytosol, nucleus.
Database links:	SwissProt: P55211 Human | Q8C3Q9 Mouse

Recommended Dilutions:

WB	1:500-1:5,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.
FC	1:1,000

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℃ or -80℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

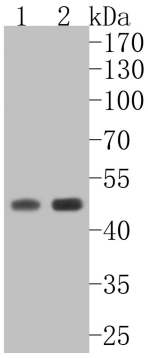

Fig1: Western blot analysis of Caspase-9 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1603-27, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: C2C12 cell lysate
Lane 2: Hela cell lysate

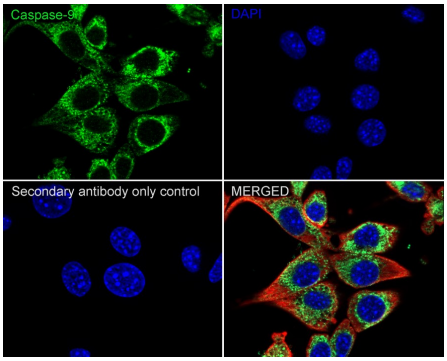

Fig2: Immunocytochemistry analysis of NIH/3T3 cells labeling Caspase-9 with Rabbit anti-Caspase-9 antibody (ET1603-27) at 1/100 dilution.

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-Caspase-9 antibody (ET1603-27) at 1/100 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.

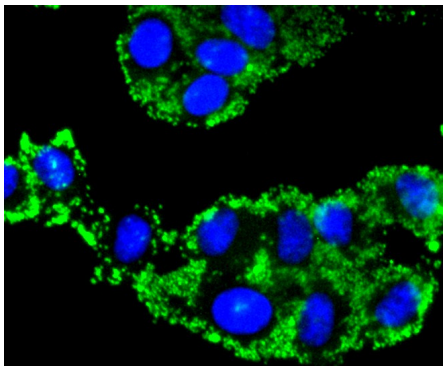

Fig3: ICC staining of Caspase-9 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-27, 1/100) for 1 hour 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).

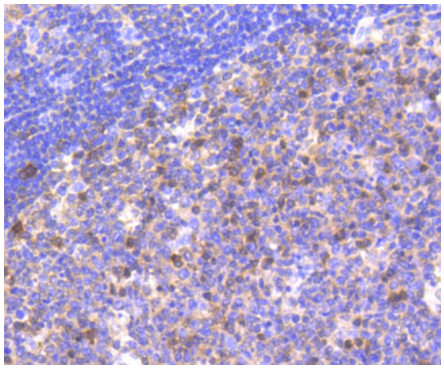

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Caspase-9 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-27, 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.

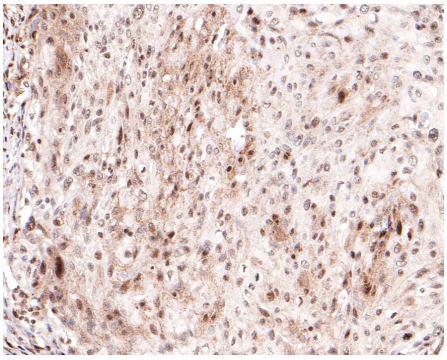

Fig5: Immunohistochemical analysis of paraffin-embedded human cervix carcinoma tissue with Rabbit anti-Caspase-9 antibody (ET1603-27) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-27) at 1/400 dilution for 1 hour 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.

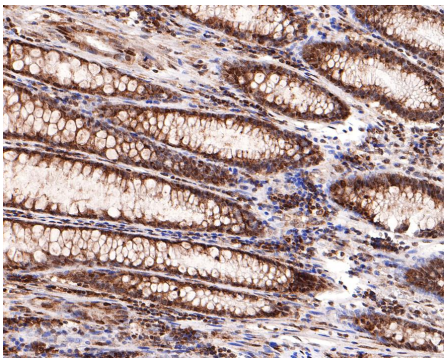

Fig6: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Caspase-9 antibody (ET1603-27) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-27) at 1/400 dilution for 1 hour 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.

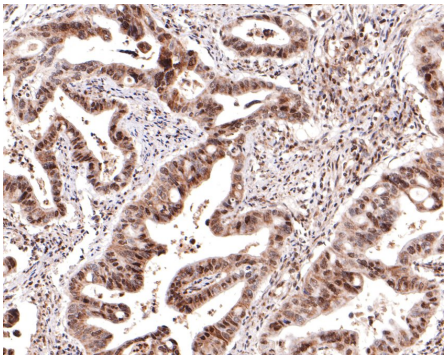

Fig7: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Caspase-9 antibody (ET1603-27) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-27) at 1/400 dilution for 1 hour 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.

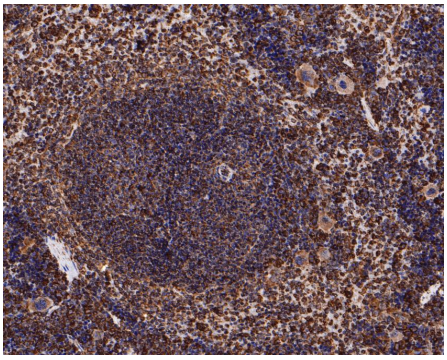

Fig8: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Caspase-9 antibody (ET1603-27) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-27) at 1/400 dilution for 1 hour 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.

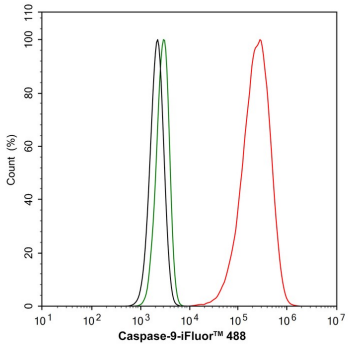

Fig9: Flow cytometric analysis of NIH/3T3 cells labeling Caspase-9.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-27, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

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. Schattenberg JM et al. Increased hepatic fibrosis and JNK2-dependent liver injury in mice exhibiting hepatocyte-specific deletion of cFLIP. Am J Physiol Gastrointest Liver Physiol 303:G498-506 (2012).