Anti-Caspase-8 Antibody [PSH04-77] - BSA and Azide free

HA750971

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 55 kDa
Clone number:	PSH04-77

Description: Caspase-8 is a caspase protein, encoded by the CASP8 gene. It most likely acts upon caspase-3. CASP8 orthologs have been identified in numerous mammals for which complete genome data are available. These unique orthologs are also present in birds. The CASP8 gene encodes a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes composed of a prodomain, a large protease subunit, and a small protease subunit. Activation of caspases requires proteolytic processing at conserved internal aspartic residues to generate a heterodimeric enzyme consisting of the large and small subunits. This protein is involved in the programmed cell death induced by Fas and various apoptotic stimuli. The N-terminal FADD-like death effector domain of this protein suggests that it may interact with Fas-interacting protein FADD. This protein was detected in the insoluble fraction of the affected brain region from Huntington disease patients but not in those from normal controls, which implicated the role in neurodegenerative diseases. Many alternatively spliced transcript variants encoding different isoforms have been described, although not all variants have had their full-length sequences determined.

Immunogen: Synthetic peptide within human Caspase-8 aa 325-374 / 479.

Positive control: Jurkat cell lysate, Jurkat treated with 25μM Etoposide for 5 hours cell lysate, Jurkat treated with 25μM Etoposide for 16 hours cell lysate, Jurkat treated with 1μM staurosporine for 3 hours cell lysate, HeLa cell lysate, HeLa treated with 1μM staurosporine for 3 hours cell lysate, HeLa treated with 100μM Etoposide for 4 hours cell lysate, human tonsil tissue, human liver tissue, HeLa cells treated with 1μM staurosporine for 3 hours.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q14790 Human

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:100
IHC-P	1:200-1:1,000
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

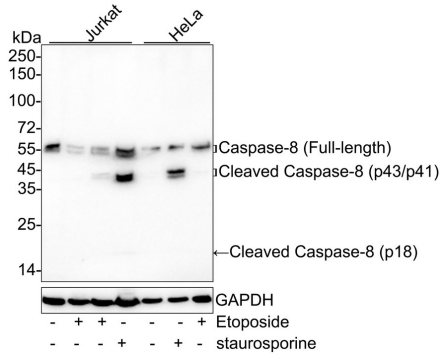

Fig1: Western blot analysis of Caspase-8 on different lysates with Rabbit anti-Caspase-8 antibody (HA750971) at 1/2,000 dilution.

Lane 1: Jurkat cell lysate (30 µg/Lane)
Lane 2: Jurkat treated with 25µM Etoposide for 5 hours cell lysate (30 µg/Lane)
Lane 3: Jurkat treated with 25µM Etoposide for 16 hours cell lysate (30 µg/Lane)
Lane 4: Jurkat treated with 1µM staurosporine for 3 hours cell lysate (30 µg/Lane)
Lane 5: HeLa cell lysate (30 µg/Lane)
Lane 6: HeLa treated with 1µM staurosporine for 3 hours cell lysate (30 µg/Lane)
Lane 7: HeLa treated with 100µM Etoposide for 4 hours cell lysate (30 µg/Lane)

Predicted band size: 55 kDa
Observed band size: 18-55 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 (HA750971) at 1/2,000 dilution was used in antibody diluent at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

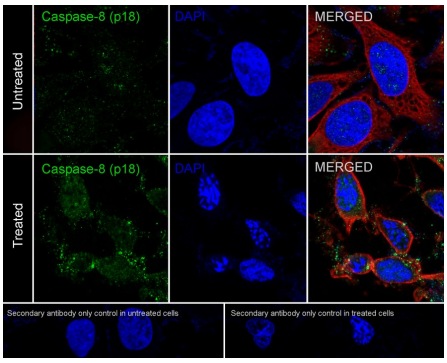

Fig2: Immunocytochemistry analysis of HeLa cells treated with or without 1µM staurosporine for 3 hours labeling Caspase-8 with Rabbit anti-Caspase-8 antibody (HA750971) at 1/100 dilution.

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-8 antibody (HA750971) 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.

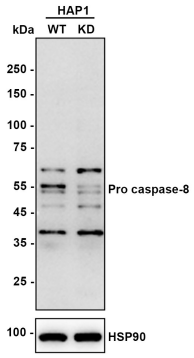

Fig3: Western blot analysis of Caspase-8 on different lysates with Rabbit anti-Caspase-8 antibody (HA750971) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Caspase-8 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 55 kDa
Observed band size: 54,55 kDa

Exposure time: 180 seconds; ECL: K1802;

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 (HA750971) 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.

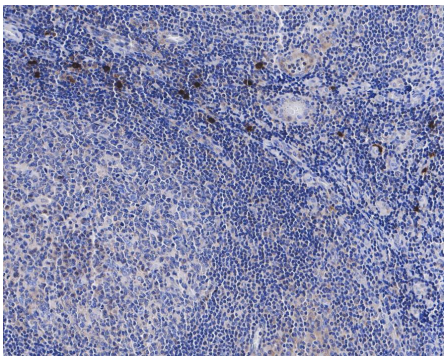

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-Caspase-8 antibody (HA750971) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (HA750971) at 1/1,000 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.

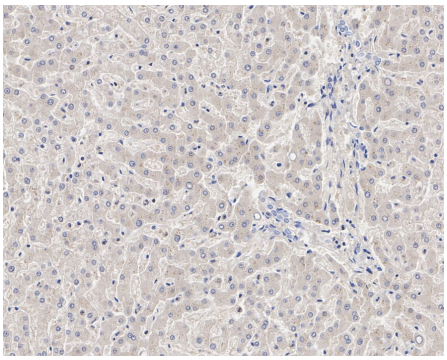

Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-Caspase-8 antibody (HA750971) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 (HA750971) at 1/200 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.

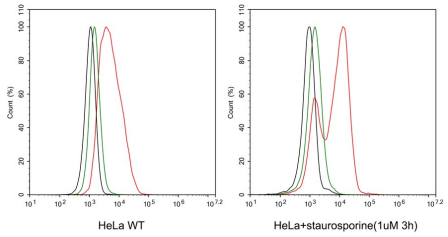

Fig6: Flow cytometric analysis of HeLa cells treated with or without 1μM staurosporine for 3 hours cells labeling Caspase-8.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750971, 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).

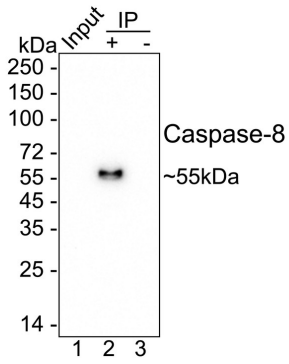

Fig7: Caspase-8 was immunoprecipitated from 0.2 mg HeLa treated with 1μM staurosporine for 3 hours cell lysate with HA750971 at 2 μg/10 μl beads. Western blot was performed from the immunoprecipitate using HA750971 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa treated with 1μM staurosporine for 3 hours cell lysate (input)
Lane 2: HA750971 IP in HeLa treated with 1μM staurosporine for 3 hours cell lysate
Lane 3: Rabbit IgG instead of HA750971 in HeLa treated with 1μM staurosporine for 3 hours cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute 28 seconds; ECL: K1801

Background References

1. Mandal R et al. Caspase-8: The double-edged sword. Biochim Biophys Acta Rev Cancer. 2020 Apr
2. Jiang M et al. Caspase-8: A key protein of cross-talk signal way in "PANoptosis" in cancer. Int J Cancer. 2021 Oct