Anti-Caspase-8 (p18) Antibody [PSH05-83] - BSA and Azide free

HA751031

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 55 kDa
Clone number:	PSH05-83

Description: Thiol protease that plays a key role in programmed cell death by acting as a molecular switch for apoptosis, necroptosis and pyroptosis, and is required to prevent tissue damage during embryonic development and adulthood. Initiator protease that induces extrinsic apoptosis by mediating cleavage and activation of effector caspases responsible for FAS/CD95-mediated and TNFRSF1A-induced cell death. Cleaves and activates effector caspases CASP3, CASP4, CASP6, CASP7, CASP9 and CASP10. Binding to the adapter molecule FADD recruits it to either receptor FAS/CD95 or TNFRSF1A. The resulting aggregate called the death-inducing signaling complex (DISC) performs CASP8 proteolytic activation. The active dimeric enzyme is then liberated from the DISC and free to activate downstream apoptotic proteases. Proteolytic fragments of the N-terminal propeptide (termed CAP3, CAP5 and CAP6) are likely retained in the DISC. In addition to extrinsic apoptosis, also acts as a negative regulator of necroptosis: acts by cleaving RIPK1 at 'Asp-325', which is crucial to inhibit RIPK1 kinase activity, limiting TNF-induced apoptosis, necroptosis and inflammatory response.

Immunogen: Synthetic peptide corresponding to residues surrounding Asp387 of mouse caspase-8 protein.

Positive control: RAW264.7 cell lysate, RAW264.7 treated with 1μM Etoposide for 18 hours cell lysate, CTLL-2 cell lysate, RAW264.7 cells treated with 1μM Etoposide for 18 hours.

Subcellular location: Cytoplasm. Nucleus.

Database links: SwissProt: O89110 Mouse

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100

Storage Buffer: 1*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

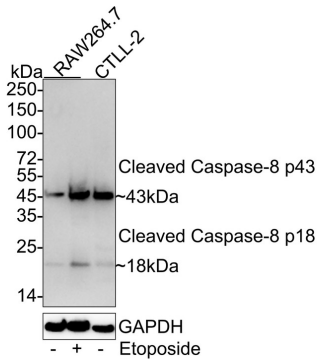

Fig1: Western blot analysis of Caspase-8 (p18) on different lysates with Rabbit anti-Caspase-8 (p18) antibody (HA751031) at 1/2,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: RAW264.7 treated with 1μM Etoposide for 18 hours cell lysate
Lane 3: CTLL-2 cell lysate

Lysates/proteins at 30 μg/Lane.

Predicted band size: 43/18 kDa
Observed band size: 43/18 kDa

Exposure time: 1 minute 20 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 (HA751031) 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.

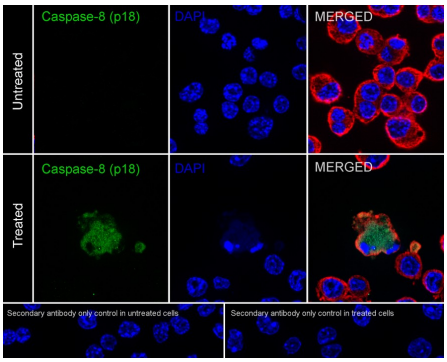

Fig2: Immunocytochemistry analysis of RAW264.7 cells treated with 1μM Etoposide for 18 hours labeling Caspase-8 (p18) with Rabbit anti-Caspase-8 (p18) antibody (HA751031) at 1/100 dilution.

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 (p18) antibody (HA751031) 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.

Background References

1. Benchoua A., Couriaud C., Guegan C., Tartier L., Couvert P., Friocourt G., Chelly J., Menissier-de Murcia J., Onteniente B. Active caspase-8 translocates into the nucleus of apoptotic cells to inactivate poly(ADP-ribose) polymerase-2. J. Biol. Chem. 277:34217-34222 (2002)
2. Newton K., Wickliffe K.E., Dugger D.L., Maltzman A., Roose-Girma M., Dohse M., Komuves L., Webster J.D., Dixit V.M. Cleavage of RIPK1 by caspase-8 is crucial for limiting apoptosis and necroptosis. Nature 574:428-431 (2019)