Human/Mouse Caspase-8 Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1650

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human/mouse Caspase-8 and cleavage products. Detects multiple isoforms of Caspase-8.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Caspase-8 Ser217-Asp384 (Asp285His) (p18 subunit), Leu385-Asp479 (p10 subunit) Accession # Q14790
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

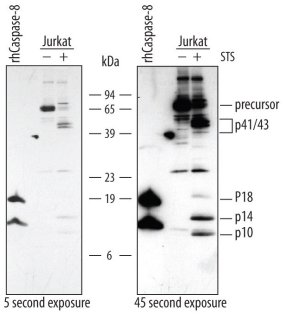
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Simple Western	5 µg/mL	See Below

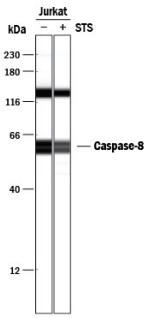
DATA

Western Blot



Detection of Human Caspase-8 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line untreated (-) or treated (+) with 1 mM staurosporine (STS) for for 3 hours. PVDF membrane was probed with 0.5 µg/mL of Rabbit Anti-Human/Mouse Caspase-8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1650), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). For additional reference Recombinant Human Caspase-8 (Catalog # 705-C8) was included. Specific bands were detected for Caspase-8 precursor at approximately 57-60 kDa (as indicated). In STS-treated samples, specific bands were detected for Caspase-8 p41/43 subunit at approximately 41 and 43 kDa (as indicated) and Caspase-8 p18, p14, and p10 subunits at approximately 18 kDa, 14 kDa, and 10 kDa, respectively (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

Simple Western



Detection of Human Caspase-8 by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line untreated (-) or treated (+) with 1 mM Staurosporine (STS) for 3 hours, loaded at 0.2 mg/mL. Specific bands were detected for Caspase-8 at approximately 58-62 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human/Mouse Caspase-8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1650). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Caspase-8 (Cysteine-aspartic acid protease 8/Casp8a; also named MCH5, FLICA and MACHα1) is a 28 kDa member of the peptidase C14A family of enzymes (1, 2, 3). It is widely expressed and is considered an initiating caspase for the apoptotic cascade (4). Caspase-8 acts on a wide variety of substrates, including procaspases-3, 4, 6, 7, 9 and 10, c-FLIP$_L$ and procaspase-8 itself (1, 5, 6). Human procaspase-8a is a 54-56 kDa, 479 amino acid (aa) protein (4, 7, 8, 9). It contains two N-terminal death domains (aa 1-177), followed by a catalytic site that utilizes His317Gly318 plus Cys360. Normally, it is an inactive, cytosolic monomer (1, 10, 11). But following death-domain (DD) containing receptor oligomerization, Caspase-8 is recruited to the death-inducing signaling complex (DISC) that forms around the death domains of the oligomerized receptor (12). FADD/CAP-1 is recruited first, followed by procaspase-8/CAP-4 and, possibly, c-FLIP$_L$ and procaspase-10 (12). The recruitment, or concentration, of procaspase-8 induces homodimerization. This act alone is sufficient for activation. However, the activity level is modest at best, and appears to be directed towards either itself, or c-FLIP$_L$, which is known to form a functional heterodimer with procaspase-8 (5, 11). When directed towards itself, autocleavage occurs first between Asp374Ser375, generating a 43 kDa (p43) N-terminal (aa 1-374) and an 11 kDa C-terminal (aa 375 - 479) fragment. The C-terminus is further cleaved between Asp384Leu385 to generate a mature p10 subunit (aa 385-479). The p43 subunit is next cleaved twice, once between Asp216Ser217, and again between Asp210Ser211 to generate a 26 kDa DD-containing prodomain (aa 1-210) with an additional 18 kDa mature p18 subunit (aa 217-374) (12). p18 and p10 noncovalently associate to form a 28 kDa heterodimer, which subsequently associates with another p18:p10 heterodimer to form an active, mature Caspase-8 molecule. This leaves the DISC to act on downstream apoptotic procaspases. In the event procaspase-8 comes to the DISC complexed with c-FLIP$_L$, c-FLIP$_L$ will be cleaved by procaspase-8, generating a p43 fragment that is analogous to the Caspase-8 p43 subunit. This fragment, however, appears not to be an intermediate in a proteolytic cascade. Rather, it serves as a functional subunit, interacting with TRAF2 and activating NFκB. This may account for many of the nonapoptotic activities associated with Caspase-8 (5, 6, 13). Mature human and mouse Caspase-8a heterodimers are 73% aa identical (14).

References:

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