Human/Mouse Caspase-8 Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF705

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse Caspase-8 in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Caspase-8 Ser234-Asp496 Accession # AAC50645
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 either lyophilized or as a 0.2 μm filtered solution in PBS.

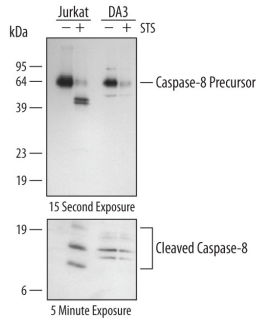
APPLICATIONS

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
Knockout Validated	Caspase-8 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in Caspase-8 knockout HeLa cell line.	

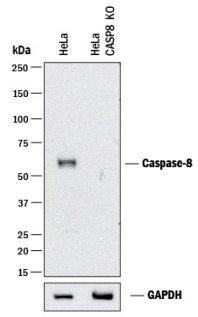
DATA

Western Blot



Detection of Human and Mouse Caspase-8 by Western Blot. Western blot shows lysates of Jurkat human leukemic T cell line and DA3 mouse myeloma cell line treated with 1 µM staurosporine for the indicated time. PVDF membrane was probed with 0.5 µg/mL Goat Anti-Human/Mouse Caspase-8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF705) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands for Caspase-8 precursor were detected at approximately 60 kDa (as indicated in upper panal) and specific bands for cleaved Caspase-8 were detected at approximately 14-18 kDa (as indicated in lower panal). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

Knockout Validated



Western Blot Shows Human Caspase-8 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and Caspase-8 knockout HeLa cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse Caspase-8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF705) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Caspase-8 at approximately 58 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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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