Human/Mouse Caspase-3 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-605-NA

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human Caspase-3.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Caspase-3 Met1-His277 Accession # AAA65015
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.

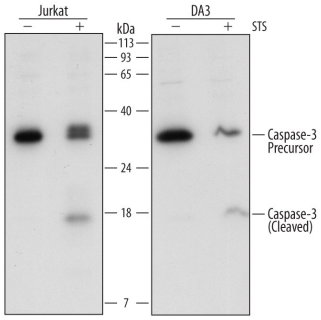
APPLICATIONS

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
Immunoprecipitation	1 µg/10^6 cells	See Below
Simple Western	5 µg/mL	See Below

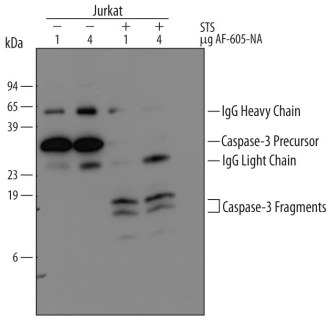
DATA

Western Blot



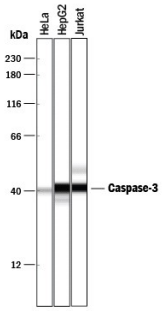
Detection of Human and Mouse Caspase-3 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line and DA3 mouse myeloma cell line untreated (-) or treated (+) with 1 µg/mL Staurosporine (STS) for 12 hours. PVDF Membrane was probed with 0.5 µg/mL of Goat Anti-Human Caspase-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-605-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for Caspase-3 precursor at approximately 34 kDa (as indicated) and Caspase-3 (cleaved) at approximately 18 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunoprecipitation



Immunoprecipitation of Human Caspase-3. Jurkat human acute T cell leukemia cell line was treated with indicated concentrations of Staurosporine for 0 or 4 hours. Caspase-3 was immunoprecipitated from lysates of 1-2 x 10^6 cells following incubation with 1 or 4 µg Goat Anti-Human Caspase-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-605-NA) overnight at 4 °C. Caspase-3-antibody complexes were absorbed using Protein G expressing Staph cells (Sigma). Immunoprecipitated Caspase-3 was detected by Western blot using 0.5 µg/mL Goat Anti-Human Caspase-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-605-NA). View our recommended buffer recipes for immunoprecipitation.

Simple Western



Detection of Human Caspase-3 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line, HepG2 human hepatocellular carcinoma cell line, and Jurkat human acute T cell leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for Caspase-3 at approximately 40 kDa (as indicated) using 5 µg/mL of Goat Anti-Human/Mouse Caspase-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-605-NA) 1:50 dilution followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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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.

BACKGROUND

Caspase-3 (Cysteine-aspartic acid protease 3/Casp3; also Yama, apopain and CPP32) is a 29 kDa member of the peptidase C14A family of enzymes (1, 2, 3). It is widely expressed and is an integral component of the apoptotic cascade. Caspase-3 is considered to be the major executioner caspase; that is, the primary downstream mediator of apoptotic-associated proteolysis (2, 3, 4). Active Caspase-3 is known to utilize a Cys residue to cleave multiple substrates, including PARP, proIL-16, PKC-γ & -δ, procaspases 6, 7 and 9, and β-catenin (1). Human procaspase-3 is a 32 kDa, 277 amino acid (aa) protein (5, 6, 7). Normally, it is an inactive, cytosolic homodimer, but following an upstream signal that activates processing proteases, procaspase-3 undergoes proteolytic cleavage (1, 2, 8, 9). This generates an N-terminal 175 aa p20/20 kDa subunit plus a 102 aa C-terminal p12/12 kDa subunit, followed by further processing of the p20 subunit at Asp28 to generate a final p17 subunit (aa 29 - 175) (9). The p17 and p12 subunits noncovalently heterodimerize, and subsequently associate with another p17/p12 heterodimer to form an active antiparallel homodimer. The p17 subunit contains the enzyme active site (aa 161 - 165), with an embedded catalytic Cys which is normally nitrosylated and inactive. Full activation requires both proteolytic processing and Cys163 denitrosylation (10). Multiple proteases can use Caspase-3 as a substrate including Caspase-6, -8, and -10, granzyme B, and Caspase-3 itself (9, 11, 12, 13).

References:

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