Human Caspase-9 Antibody

Monoclonal Mouse IgG_1 Clone # LAP6

Catalog Number: MAB8301

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Caspase-9 in Western blots and captures Caspase-9 complexed with APAF-1.
Source	Monoclonal Mouse IgG_1 Clone # LAP6
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human Caspase-9 aa 1-134
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	1 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	Immersion fixed paraffin-embedded sections of human colon
Simple Western	20 µg/mL	See Below

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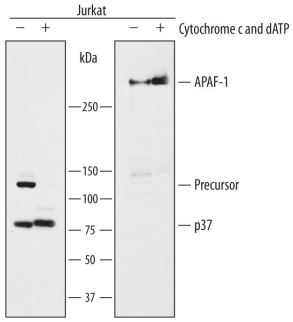
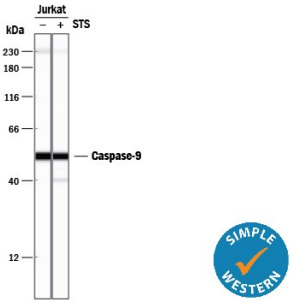
DATA

Western Blot



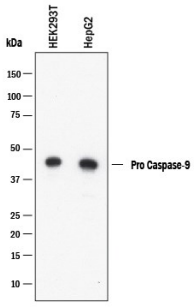
Capture of Human Caspase-9 and Human Caspase-9 complexed with APAF-1 detected by Western Blot. Western blot shows Jurkat human acute T cell leukemia cell line lysates untreated (-) or treated (+) with 50 mM dATP and 1 mg/mL rat cytochrome c for 60 minutes, then captured on a 6-well dish coated at 10 µg/mL with Mouse Anti-Human Caspase-9 Monoclonal Antibody (Catalog # MAB8301). PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Caspase-9 Monoclonal Antibody (Catalog # MAB8301, left side) or Mouse Anti-Human APAF-1 Monoclonal Antibody (Catalog # MAB868, right side) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Specific bands were detected for Caspase-9 Precursor at approximately 46 kDa and the Caspase-9 p37 subunit at approximately 37 kDa (as indicated). A specific band was detected for APAF-1, captured as part of Caspase-9 complexed with APAF-1, at approximately 135 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

Simple Western



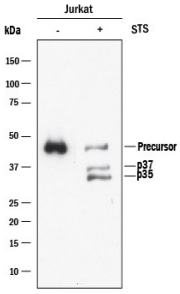
Detection of Human Caspase-9 by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line untreated (-) or treated (+) with 1 mM Staurosporine (STS) for 3 hours, loaded at 0.2 mg/mL. A specific band was detected for Caspase-9 at approximately 53 kDa (as indicated) using 20 µg/mL of Mouse Anti-Human Caspase-9 Monoclonal Antibody (Catalog # MAB8301). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system. Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

Western Blot



Detection of Human Caspase-9 by Western Blot. Western blot shows lysates of HEK293T human embryonic kidney cell line and HepG2 human hepatocellular carcinoma cell line. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Caspase-9 Monoclonal Antibody (Catalog # MAB8301) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Caspase-9 at approximately 46 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 4.

Western Blot



Detection of Human Caspase-9 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line untreated (-) or treated (+) with 1 ug/ml Staurosporine (STS) for 2 hours. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Caspase-9 Monoclonal Antibody (Catalog # MAB8301) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Caspase-9 at approximately 46, 37, 35 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 4.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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-9 (Cysteine-aspartic acid protease 9/Casp-9; also APAF-3, Mch6 and ICE-LAP6) is a 35-37 kDa member of the peptidase C14A family of enzymes. Casp-9 is an initiator caspase that is part of the intrinsic apoptosis pathway. It is widely expressed and is particularly important during development. Human proCaspase-9 is a 47-48 kDa, 416 amino acid (aa) protein and it contains one CARD region (aa 1-92) and catalytic residues at His237 and Cys287. Following mitochondrial disruption, cytochrome c is released from mitochrondria. Cytochrome c acts on APAF-1, which induces procaspase-9 dimerization. The act of dimerization activates proCasp-9, leading to either the activation of Casp-3, or the autocleavage of proCasp-9, generating a 35 kDa subunit (aa 1-315) and a 12 kDa subunit. Activated Casp-3 will also act on proCasp-9, generating a 37 kDa subunit (aa 1-330) and a 10 kDa subunit (aa 331-416). These subunits associate to form an active heterotetramer. Casp-9 has an alternative start site at Met84 and a deletion of aa 140-289 that generates a dominant negative, 31 kDa isoform. Over aa 1-134, human Casp-9 shares 81% aa identity with mouse Casp-9.

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