

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	<i>E. coli</i> -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 as a 0.2 µm filtered solution in PBS.

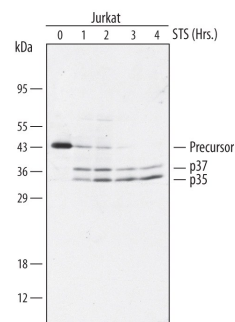
APPLICATIONS

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

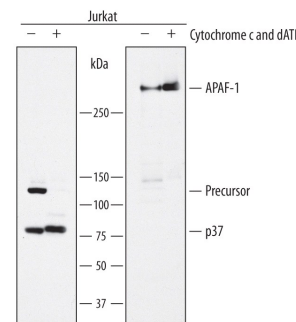
DATA

Western Blot



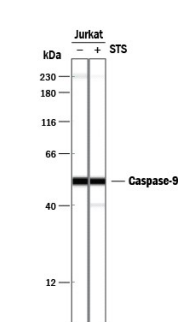
Detection of Human Caspase-9 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line treated with 1 µg/mL staurosporine (STS) for the indicated times. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Caspase-9 Monoclonal Antibody (Catalog # MAB8301) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Specific bands were detected for Caspase-9 Precursor at approximately 46 kDa and the Caspase-9 p37 and p35 subunits at approximately 37 kDa and 35 kDa, respectively (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

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, 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.

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. <ul style="list-style-type: none">● 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-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 mitochondria. 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.