

Human/Mouse Cleaved Caspase-3 (Asp175) Antibody

Monoclonal Rabbit IgG Clone # 269518 Catalog Number: MAB835

DESCRIPTION			
Species Reactivity	Human/Mouse		
Specificity	Detects human and mouse Caspase-3 cleaved at Asp175. No cross-reactivity was detected with the full-length procaspase-3 or other caspases.		
Source	Monoclonal Rabbit IgG Clone # 269518		
Purification	Protein A or G purified from hybridoma culture supernatant		
Immunogen	KLH-conjugated human Caspase-3 synthetic peptide CRGTELDCGIETD Accession # U26943		
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
Immunocytochemistry	8-25 μg/mL	See Below
Immunohistochemistry	0.3-25 μg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below

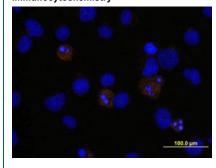
DATA

Western Blot

Detection of Human and Mouse Cleaved Caspase-3 (Asp175) by Western Blot Western blot shows

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 µM staurosporine (STS) for the indicated times. PVDF membrane was probed with 0.5 µg/mL of Human/Mouse Cleaved Caspase-3 (Asp175) Monoclonal Antibody (MAB835), followed by HRPconjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Cleaved Caspase-3 (Asp175) at approximately 18 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.

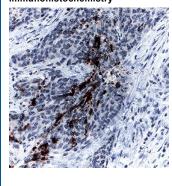
Immunocytochemistry



Caspase-3 in Jurkat Human

Cell Line. Caspase-3 was detected in immersion fixed Jurkat human acute T cell leukemia cell line treated with staurosporin using Human/Mouse Cleaved Caspase-3 (Asp175) Monoclonal Antibody (Catalog # MAB835) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent

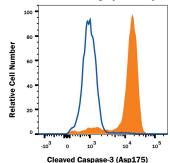
Immunohistochemistry



Caspase-3 in Human Colon Cancer Tissue. Caspase-3 was detected in immersion fixed paraffin-embedded sections of human colon cancer tissue using Rabbit Anti-Human/Mouse Cleaved Caspase-3 (Asp175) Monoclonal Antibody (Catalog # MAB835) at 0.3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm. View our protocol for IHC Staining with

VisUCyte HRP Polymer Detection Reagents

Intracellular Staining by Flow Cytometry



Detection of Cleaved Caspase-3 in Jurkat Human Cell Line by Flow Cytometry. Jurkat human acute T cell leukemia cell line untreated (open histogram) or treated with 3 µM Staurosporine for 3 hours (filled histogram) was stained with Rabbit Anti-Human/Mouse Caspase-3 Monoclonal Antibody (Catalog # MAB835, filled histogram) followed by anti-Rabbit IgG FITC-conjugated secondary antibody (Catalog # F0112). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with 90% methanol. View our protocol for Staining Intracellular Molecules.

Rev. 10/1/2019 Page 1 of 2





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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.		

BACKGROUND

Caspase-3 (Cysteine-aspartic acid protease 3/Casp3; also Yama, apopain and CPP32) is a 29 kDa heterodimer that belongs to the peptidase C14A family of enzymes. It is widely expressed and considered to be the major executioner caspase in the apoptotic cascade. Human procaspase-3 is a 32 kDa, 277 amino acid (aa) protein and is normally an inactive homodimer. Following cell stress/activation, procaspase-3 undergoes proteolysis to generate an N-terminal 148 aa p17/17 kDa subunit (aa 29-175), plus a 102 aa C-terminal p12/12 kDa subunit. These subunits noncovalently heterodimerize, and associate with another p17/p12 heterodimer to form an active enzyme. There is one potential variant that shows an alternative start site nine aa upstream of the standard start site coupled with a 21 aa substitution for aa 162-277. Over aa 29-175, human and mouse caspase-3 share 87% aa identity.



