

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 CRGTELDGCIETD 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 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 HRP-conjugated 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 staurosporine 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™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

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](#).

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-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.