

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Distinguishes apoptotic cells from non-apoptotic cells in immunohistochemistry. Detects the p17 subunit of Caspase-3, but does not detect the precursor form in Western blot. We recommend R&D Systems Affinity Purified Anti-Caspase-3 (Catalog # AF-605-NA) or Monoclonal Anti-Cleaved-Caspase-3 (Catalog # MAB835) for Western blot applications.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	KLH-coupled human Caspase-3 synthetic peptide CRGTELDGCIETD Accession # P42574
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.

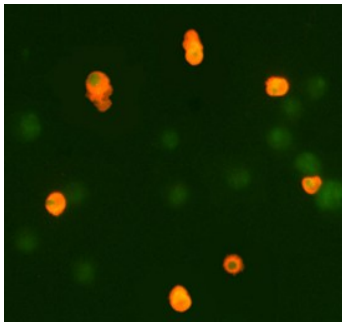
APPLICATIONS

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
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	1-15 µg/mL	See Below

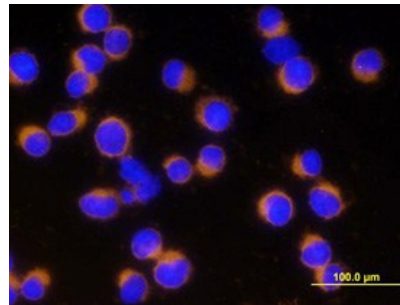
DATA

Immunocytochemistry



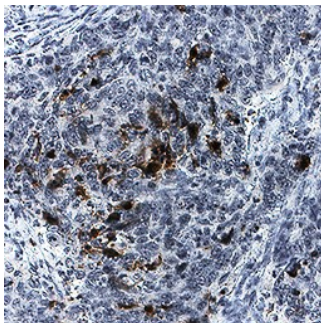
Caspase-3 in Jurkat Human Cell Line. Caspase-3 was detected in immersion fixed anti-FAS treated Jurkat human acute T cell leukemia cell line using 0.3 µg/mL Human/Mouse Active Caspase-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF835) for 3 hours at room temperature. Cells were stained (red) and counterstained (green). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



Caspase-3 in Jurkat Human Cell Line. Caspase-3 was detected in immersion fixed Jurkat human acute T cell leukemia cell line stimulated with staurosporin using Human/Mouse Active Caspase-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF835) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (yellow; Catalog # NL004) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

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 Active Caspase-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF835) at 1 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](#).

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. <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.