CASP3 [D175] ABfinity™ Recombinant Rabbit Monoclonal Antibody - Purified

REF Catalog no. 700182

(See product label for lot information)

Clone/PAD: 9H19L2
Isotype: IgG
Gene ID: 836
Protein Acc. No.: P42574
Qty: 100 µg
Volume: 200 µl
Concentration: 0.5 mg/ml

Formulation
PBS + 0.09% azide

Immunogen
A peptide corresponding to amino acids 171-175 of P42574.

Immunogen sequence
GIETD

Reactivity
This antibody reacts with human CASP3 [D175]. Based on sequence identity and similarity, reactivity to mouse, rat, primate, hamster, canine, bovine, feline, swine, rabbit, pufferfish, and Xenopus is expected.

Specificity
This antibody is specific for the cleaved (active) form of caspase 3

Storage
2-8°C for up to 1 mo, -20°C for long term storage. Avoid repeated freezing and thawing.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Validated Applications:

	Species	Test Material	Concentration
Western Blotting	human	Jurkat + staurosporine	0.1-0.2 µg/ml
Immunohistochemistry	human	normal tonsil	1-2 µg/ml
Immunofluorescence	human	A549 + staurosporine	5-6 µg/ml
Flow Cytometry	human	Jurkat + camptothecin	0.5-1 µg/test

Background
Caspases are a family of cysteine proteases that centrally controls apoptotic machinery (1). Caspases can be grouped according to their substrate specificities that are largely determined by the amino acids preceding the cleavage site. One group of caspases that include -6, -8, (MACH/FLICE), and –9(V/LEXD) is specific for the substrate V/LEXD. This substrate is a site similar to those found in caspase proenzymes. This group of caspases may function as initiators of a proteolytic cascade by activating pro-caspases to amplify a death signal. A second group of caspases (-2, -3 and –7) is specific for the substrate DEXD that is related to sites found on target proteins cleaved during apoptosis.
Caspase-3 (also known as CPP32, Yama or Apopain) is a member of the interleukin-1β converting enzyme (ICE) family of cysteine proteases (4). Caspase-3 exists in cells as an inactive 32 kDa proenzyme, called pro-Caspase-3. Pro-Caspase-3 is cleaved into active 17 and 12 kDa subunits by upstream proteases such as Caspase-6 (Mch2), Caspase-8 (FLICE) and Granzyme B during apoptosis (2). The downstream substrates of Caspase-3 include poly-ADP ribose polymerase (PARP), sterol regulatory element binding proteins (SREBPs), nuclear lamins and others (5). The overexpression of Caspase-3 can result in apoptosis (4). Likewise, the inhibition of Caspase-3 or other caspases can prevent cells from entering the apoptotic pathway (6). Recent evidence has revealed a link between plasma caspase-3 and atherosclerosis (7) and it role in activation of apoptosis in breast cancer mediated by siRNA-mediated Apollon silencing (8). This antibody is specific for the cleaved (active) form of caspase-3.

References

1. Stennicke H.R. and Salvensen G.S. (1998) Properties of the caspases. Biochim. Biophy. Acta. 1387:17-31.
2. Anel A., et al. (1997) Inhibition of CPP32-like proteases prevents granzyme B- and Fas-, but not granzyme A-based cytotoxicity exerted by CTL clones. J. Immunol. 158:1999-2006.
3. Casciola-Rosen, L.A., et al. (1996) Apopain/CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. J. Exp. Med. 183:1957-1964.
4. Jaeschke H., et al. (1998) Activation of caspase 3 (CPP32)-like proteases is essential for TNF-α induced hepatic parenchymal cell apoptosis and neutrophil-mediated necrosis in a murine endotoxin shock model. J. Immunol. 160:3480-3486.
5. Lazebnik Y., et al. (1994) Cleavage of poly (ADP-ribose) polymerase by a proteinase with properties like ICE. Nature 371:346-347.
6. Martin, D.S.D., et al. (2002) Apoptotic changes in the aged brain are triggered by interleukin-1 bet a-induced activation of p38 and reversed by treatment with eicosapentaenoic acid. J. Biol. Chem. 277:34239-34246.
7. Matulevicius, S., et al. (2008) The association between plasma caspase-3, atherosclerosis, and vascular function in the Dallas Heart Study. Apoptosis 13:1281-1289.
8. Lopergolo, A., et al. (2009) Apollon gene silencing induces apoptosis in breast cancer cells through p53 stabilisation and caspase-3 activation. Br. J. Cancer 11:739-746.

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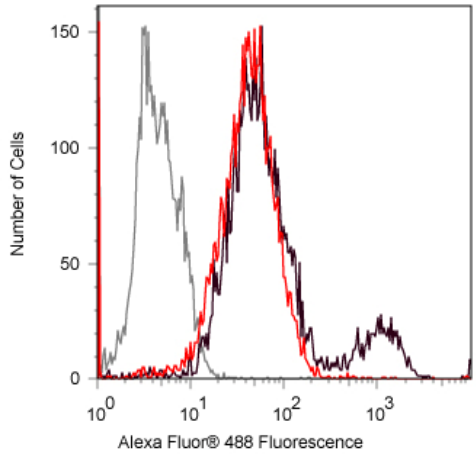
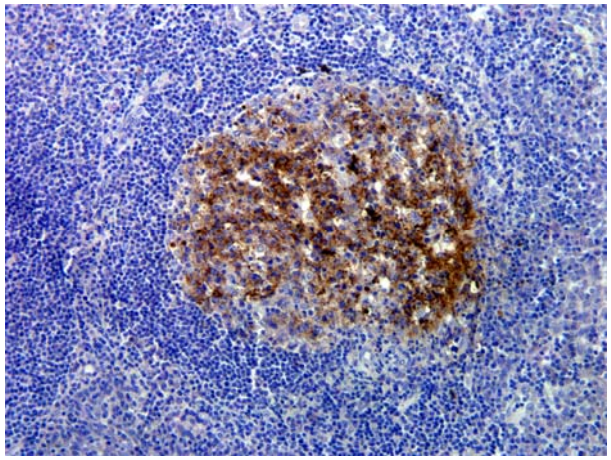


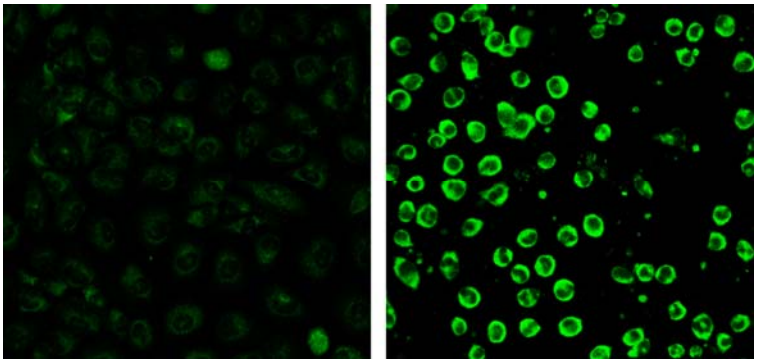
Flow cytometry of Jurkat cells labeled with rabbit anti-CASP3 [D175] (Cat. No. 700182).

Jurkat cells were incubated in the absence (red trace) or presence (black trace) of 10 µM camptothecin for 4 h prior to being fixed and permeabilized using FIX & PERM® reagents (Cat. No. GAS004). Cells were then stained with 0.5 µg anti-CASP3 [D175] followed by Alexa Fluor® 488 goat anti-rabbit Ig (Cat. No. A11008). The gray trace represents cells stained with secondary antibody only.



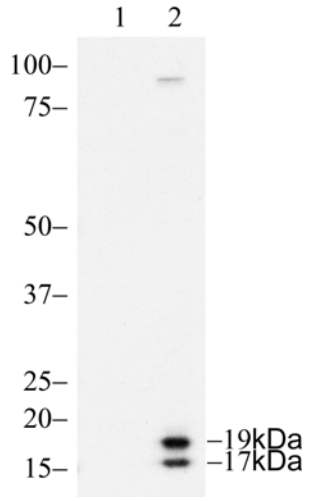
Immunohistochemistry of human tonsil tissues labeled with rabbit anti-CASP3 [D175] (Cat. No. 700182).

FFPE human normal tonsil tissue was labeled with rabbit anti-CASP3 [D175] (1 µg/ml). Tissues were detected with SuperPicTure™ Polymer DAB (Cat. No.87-8963). Images were taken at 20x magnification. Note cytoplasmic staining of proliferating cells in the germinal center area.



Immunocytochemistry of A549 cells labeled with rabbit anti-CASP3 [D175] (Cat. No. 700182).

A549 cells labeled with rabbit anti-CASP3 [D175] (5 µg/ml) treated without (left) or with (right) staurosporine . Alexa Fluor® 488 goat anti-rabbit (Cat. No. A11008) at 1:1000 was used as secondary antibody.



Western blot of Jurkat lysates labeled with rabbit anti-CASP3 [D175] (Cat. No. 700182).

Rabbit anti-CASP3 [D175] (0.1 µg/ml) was used to label cleaved caspase 3 in untreated Jurkat lysates (lane 1) or Staurosporin treated Jurkat lysates (lane 2).