abcam

ab32150

Anti-pro Caspase-3 antibody [E61]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	E61
Purification technique	Affinity purification Protein A
Specificity	The antibody only recognizes the pro-form of Caspase-3. It does not react with the cleaved forms (active enzyme) of Caspase-3.
Concentration	0.134 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/500

Notes	-

IP

Tested

Species	Human
Dilution info	1/20
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/200
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information CASP3

Function	Thiol protease that acts as a major effector caspase involved in the execution phase of apoptosis (PubMed:18723680, PubMed:20566630, PubMed:23650375, PubMed:35338844, PubMed:35446120, PubMed:7596430). Following cleavage and activation by initiator caspases (CASP8, CASP9 and/or CASP10), mediates execution of apoptosis by catalyzing cleavage of many proteins (PubMed:18723680, PubMed:20566630, PubMed:23650375, PubMed:7596430). At the onset of apoptosis, it proteolytically cleaves poly(ADP-ribose) polymerase PARP1 at a '216-Asp-|-Gly-217' bond (PubMed:10497198, PubMed:16374543, PubMed:7596430, PubMed:7774019). Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain (By similarity). Cleaves and activates caspase-6, -7 and -9 (CASP6, CASP7 and CASP9, respectively) (PubMed:7596430). Cleaves and inactivates interleukin-18 (IL18) (PubMed:37993714, PubMed:9334240). Involved in the cleavage of huntingtin (PubMed:8696339). Triggers cell adhesion in sympathetic neurons through RET cleavage (PubMed:21357690). Cleaves DSG2 in response to apoptosis resulting in a loss of full length DSG2 at desmosome cell junctions and subsequent loss of cell-cell adhesion (PubMed:17559062). Also cleaves JUP in response to apoptosis (PubMed:17559062). Cleaves and inhibits serine/threonine-protein kinase AKT1 in response to oxidative stress (PubMed:23152800). Acts as an inhibitor of type I interferon production during virus-induced apoptosis by mediating cleavage of antiviral proteins CGAS, IRF3 and MAVS, thereby preventing cytokine overproduction (PubMed:30878284). Also involved in pyroptosis by mediating cleavage and activation of gasdermin-E (GSDME) (PubMed:35338844, PubMed:35446120). Cleaves XRCC4 and phospholipid scramblase proteins XKR4, XKR8 and XKR9, leading to promote phosphatidylserine exposure on apoptotic cell surface (PubMed:23845944, PubMed:33725486). Cleaves BIRC6 following inhibition of BIRC6-caspase binding by DIABLO/SMAC (PubMed:36758104, PubMed:36758106).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Species reactivity
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Pro Caspase-3 also known as Caspase-3 zymogen is a central component in the apoptotic process. This inactive precursor form undergoes cleavage during apoptosis to become active caspase-3 an important executioner in cell death. Caspase-3 possesses a molecular weight of approximately 32 kDa. It is expressed ubiquitously in cells throughout the body with a higher concentration in tissues with rapid cell turnover such as thymus and gastrointestinal tract.
Biological function summary	The inactive pro Caspase-3 form serves as a safeguard in cells preventing premature apoptosis. It only becomes the active caspase-3 after specific activation ensuring controlled progression of apoptosis. This inactive form does not participate in any complex but becomes a part of the caspase family network upon cleavage. In its active form caspase-3 leads to the cleavage of several key cellular proteins driving the morphological and biochemical changes seen during apoptosis.
Pathways	Pro Caspase-3 is critical in the apoptotic signaling pathway. It serves as a link between intrinsic and extrinsic pathways of apoptosis. The intrinsic pathway often triggered by mitochondrial signals and the extrinsic pathway initiated by death receptor signals both lead to the activation of caspase-3. In these pathways pro Caspase-3 interacts with proteins like caspase-8 and cytochrome c playing a part in their signaling cascades and cellular outcomes.
Associated diseases and disorders	Pro Caspase-3 is associated with conditions involving dysregulated apoptosis. One such condition is cancer where evasion of apoptosis leads to uncontrolled cell proliferation. The therapeutic targeting of pro Caspase-3 activation holds potential in reinstating apoptotic pathways in cancerous cells. Additionally it relates to neurodegenerative diseases like Alzheimer's where excessive apoptosis contributes to neuronal loss. Here active caspase-3 interacts with proteins like amyloid precursor protein suggesting a role in the disease's progression.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

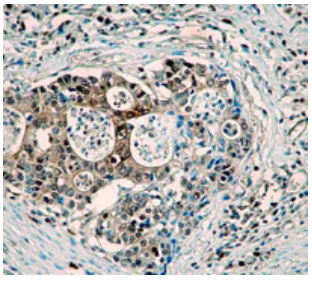
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

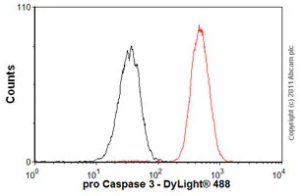
7 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-pro Caspase-3 antibody [E61] (ab32150)

Pro Caspase-3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-pro Caspase-3 antibody

Immunohistochemical analysis of human paraffin-embedded cervical carcinoma tissue using ab32150 at 1/500 dilution.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

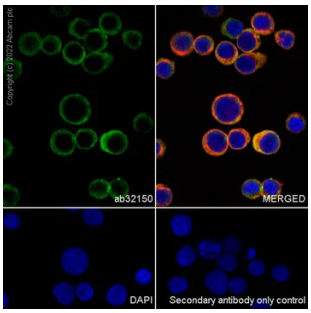


Flow Cytometry (Intracellular) - Anti-pro Caspase-3 antibody [E61] (ab32150)

Pro Caspase-3 Flow Cytometry (Intracellular) staining using rabbit Anti-pro Caspase-3 antibody

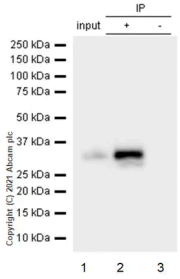
Overlay histogram showing Jurkat cells stained with ab32150 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32150, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was anti-rabbit DyLight® 488 (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a

positive signal in Jurkat cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-pro Caspase-3 antibody [E61] (ab32150)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cell line from peripheral blood) cells labelling pro Caspase-3 with primary antibody anti-pro Caspase-3 (ab32150) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150081) secondary antibody at 1/1000 dilution. Confocal image showing cytoplasmic staining in Jurkat cells. Anti-alpha Tubulin antibody (DM1A) - Microtubule Marker (Alexa Fluor® 594) (ab195889) was used to counterstain tubulin at 1/200 dilution. The nuclear counter stain is DAPI (blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunoprecipitation - Anti-pro Caspase-3 antibody [E61] (ab32150)

pro Caspase-3 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with ab32150 at 1/20 dilution (0.6µg). VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg

Lane 2: ab32150 IP in HeLa whole cell lysate

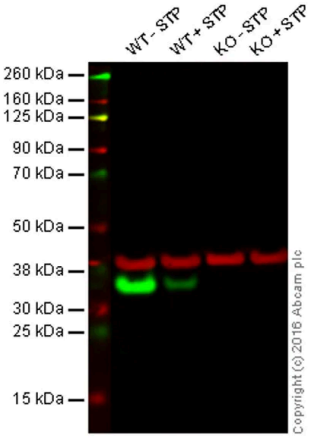
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab32150 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-pro Caspase-3 antibody [E61] (ab32150)

Predicted band size: 31 kDa

Observed band size: 35 kDa



Western blot - Anti-pro Caspase-3 antibody [E61] (ab32150)

Pro Caspase-3 Western blot staining using rabbit Anti-pro Caspase-3 antibody

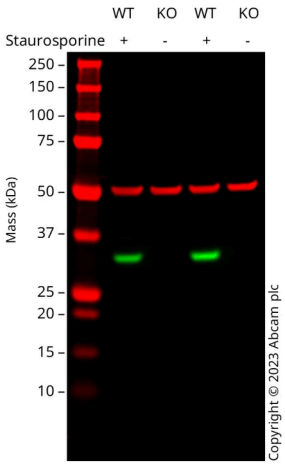
Lane 1: Wild-type HAP1 cell lysate
Lane 2: Wild-type HAP1 cell lysate + Staurosporine (1µM for 4h)
Lane 3: Caspase-3 knockout HAP1 cell lysate
Lane 4: Caspase-3 knockout HAP1 cell lysate + Staurosporine (1µM for 4h)
Lanes 1 - 4: Merged signal (red and green). Green - ab32150 observed at 35 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab32150 was shown to specifically react with pro Caspase 3 when Caspase 3 knockout samples were used. Wild-type and Caspase 3 knockout samples (± Staurosporine treatment) were subjected to SDS-PAGE. ab32150 and ab8245 (loading control to GAPDH) were diluted to 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-pro Caspase-3 antibody [E61] (ab32150)

Predicted band size: 31 kDa

Observed band size: 35 kDa

Western blot - Anti-pro Caspase-3 antibody [E61] (ab32150)



Anti-CASP3 antibody [E61] (ab32150) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32150 was shown to bind specifically to CASP3. A band was observed at 30 kDa in treated wild-type HeLa cell lysates with no signal observed at this size in CASP3 knockout cell line. To generate this image, wild-type and CASP3 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-pro Caspase-3 antibody [E61] (ab32150) at 1/500 dilution

Lane 1:
Wild-type HeLa Treated Staurosporine (2uM, 4h) cell lysate at 20 µg

Lane 2:
CASP3 knockout HeLa Treated Staurosporine (2uM, 4h) cell lysate at 20 µg

Lane 3:
Wild-type HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate at 20 µg

Lane 4:
CASP3 knockout HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate at 20 µg

Secondary

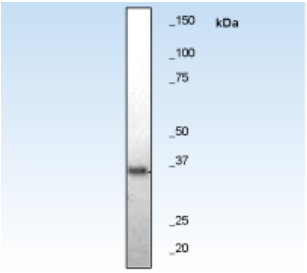
Lanes 1 - 4:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 4:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 31 kDa

Observed band size: 30 kDa



Western blot - Anti-pro Caspase-3 antibody [E61] (ab32150)

All lanes:
Western blot - Anti-pro Caspase-3 antibody [E61] (ab32150) at 1/1000 dilution

All lanes:
Hela cell lysate

Predicted band size: 31 kDa

Observed band size: 35 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.