abcam

Product datasheet

Anti-Caspase-3 antibody [E87] ab32351

KO VALIDATED Recombinant RabMAb

14 Abreviews 299 References 12 Images

Overview

Product name	Anti-Caspase-3 antibody [E87]
Description	Rabbit monoclonal [E87] to Caspase-3
Host species	Rabbit
Specificity	This antibody is specific for the pro form and the p17 cleaved form of human Caspase-3.
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), WB, IHC-P, IP
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse
Immunogen	Synthetic peptide within Human Caspase-3 aa 50-150. The exact sequence is proprietary. Database link: **P42574**
Positive control	WB: Jurkat whole cell lysate (**ab7899**); Wild-type HAP1 whole cell lysate; Ramos and HEK-293 cell lysates. IHC-P: Human tonsil and cervical carcinoma tissue. ICC/IF: Jurkat cells and wild-type HAP1 cells. Flow Cyt (intra): HeLa and Ramos cells. IP: HeLa whole cell lysate.
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E87
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32351 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★☆☆ (3)	Use a concentration of 1 µg/ml. **For unpurified, use 1/25 dilution.**
Flow Cyt (Intra)		1/180 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (7)	1/5000. Detects a band of approximately 35 kDa (predicted molecular weight: 32 kDa).
IHC-P	★★★★☆ (2)	1/25 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IP		1/10 - 1/50.

Target

Function Involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis it proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp--Gly-217' bond. Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Cleaves and activates caspase-6, -7 and -9. Involved in the cleavage of huntingtin.

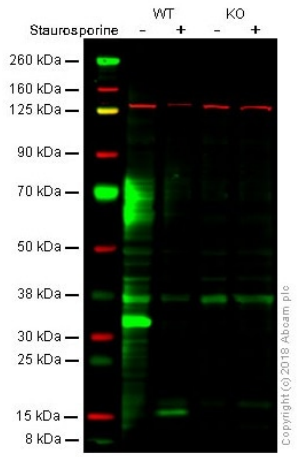
Tissue specificity Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system.

Sequence similarities Belongs to the peptidase C14A family.

Post-translational modifications Cleavage by granzyme B, caspase-6, caspase-8 and caspase-10 generates the two active subunits. Additional processing of the propeptides is likely due to the autocatalytic activity of the activated protease. Active heterodimers between the small subunit of caspase-7 protease and the large subunit of caspase-3 also occur and vice versa.
S-nitrosylated on its catalytic site cysteine in unstimulated human cell lines and denitrosylated upon activation of the Fas apoptotic pathway, associated with an increase in intracellular caspase activity. Fas therefore activates caspase-3 not only by inducing the cleavage of the caspase

	zymogen to its active subunits, but also by stimulating the denitrosylation of its active site thiol.
Cellular localization	Cytoplasm.

Images



Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

All lanes : Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution

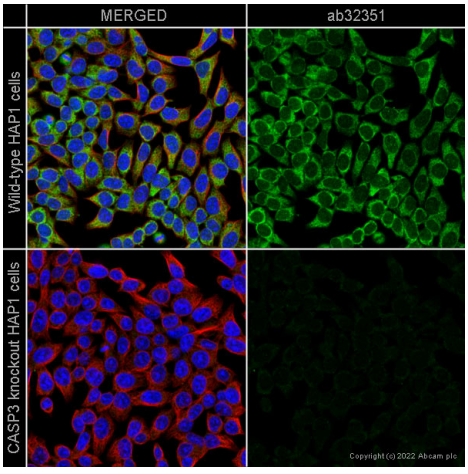
Lane 1 : DMSO control wild-type HAP1 whole cell lysate
Lane 2 : Staurosporine treated wild-type HAP1 whole cell lysate
Lane 3 : DMSO control CASP3 knockout HAP1 whole cell lysate
Lane 4 : Staurosporine treated CASP3 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 32 kDa

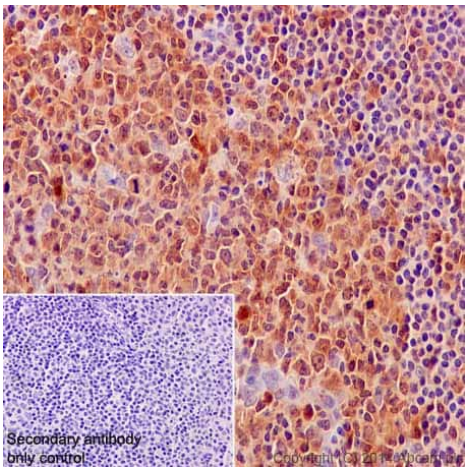
Lanes 1 - 4: Merged signal (red and green). Green - ab32351 observed at 31 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

ab32351 was shown to recognize Caspase 3 in wild-type HAP1 cells as signal was lost at the expected MW in HAP1 Staurosporine Treated (CASP3) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and HAP1 Staurosporine Treated (CASP3) knockout samples were subjected to SDS-PAGE. ab32351 and **ab130007** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



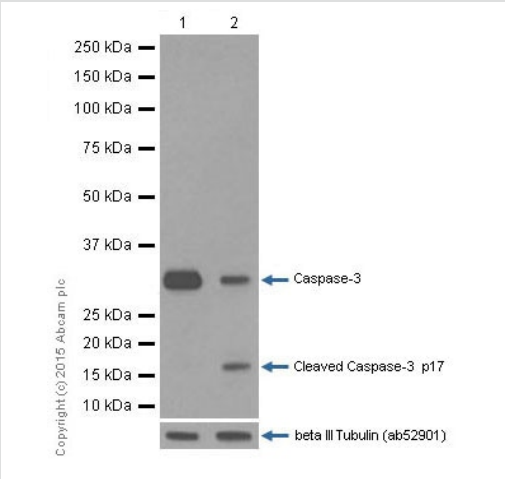
Immunocytochemistry/ Immunofluorescence - Anti-Caspase-3 antibody [E87] (ab32351)

ab32351 staining Caspase-3 in wild-type Hap1 cells (top panel) and CASP3 knockout Hap1 cells (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab32351 at 1µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [E87] (ab32351)

Immunohistochemical staining of paraffin embedded human tonsil with purified ab32351 at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

All lanes : Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution (purified)

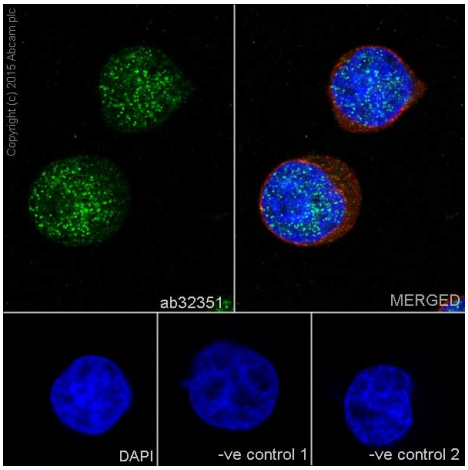
Lane 1 : untreated Jurkat (human T cell leukemia cell line from peripheral blood)cell lysate
Lane 2 : Jurkat treated with staurosporine

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

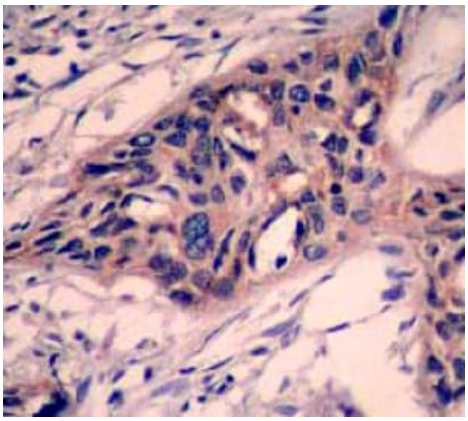
Predicted band size: 32 kDa
Observed band size: 17,35 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-3 antibody [E87] (ab32351)

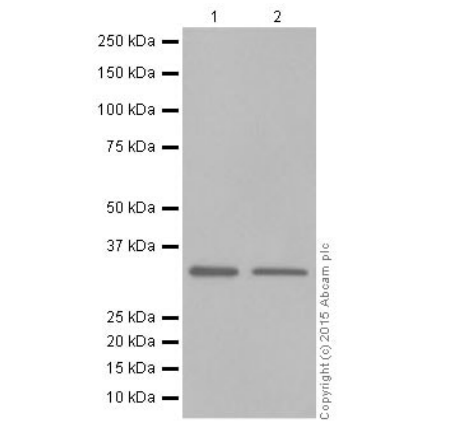
Immunofluorescence staining of Jurkat (human T cell leukemia cell line from peripheral blood) cells with purified ab32351 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab32351 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [E87] (ab32351)

Unpurified ab32351, at a 1/25 dilution, staining Capase-3 in paraffin embedded human cervical carcinoma tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

All lanes : Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution (purified)

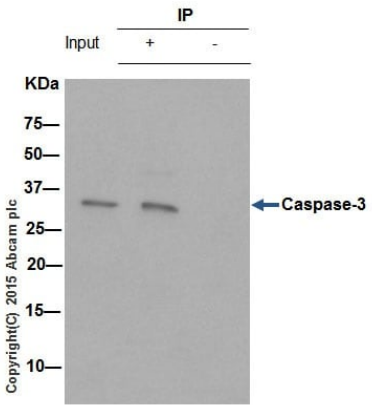
Lane 1 : Ramos (human Burkitt's lymphoma cell line) cell lysate
Lane 2 : HEK-293 (human epithelial cell line from embryonic kidney) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

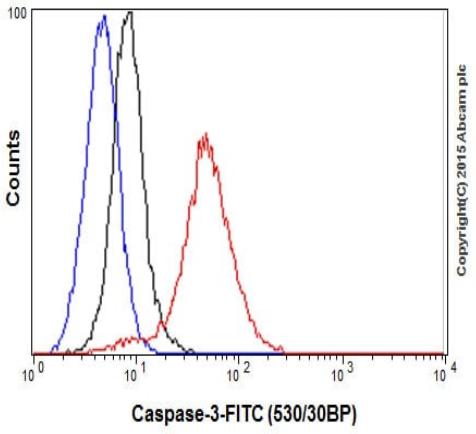
Predicted band size: 32 kDa
Observed band size: 35 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



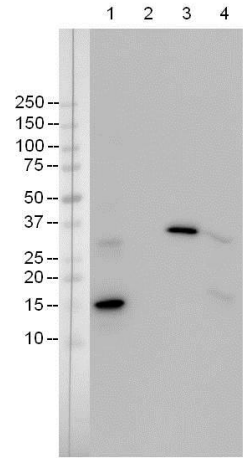
Immunoprecipitation - Anti-Caspase-3 antibody [E87] (ab32351)

ab32351 (purified) at 1/50 immunoprecipitating Cullin 1 in 10 µg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate (Lanes 1 and 2, observed at 35 kDa). Lane 3 - PBS. For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection (1/1500). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



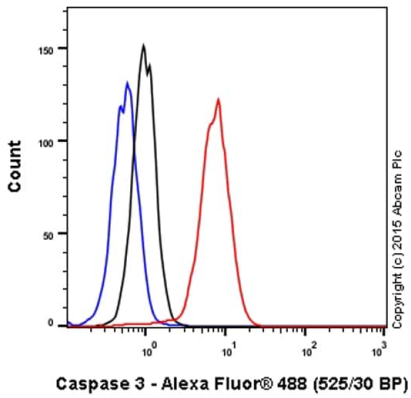
Flow Cytometry (Intracellular) - Anti-Caspase-3 antibody [E87] (ab32351)

Overlay histogram showing Ramos (human Burkitt's lymphoma cell line) cells fixed in 4% PFA and stained with purified ab32351 at a dilution of 1 in 180 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



Western blot - Anti-Caspase-3 antibody [E87] (ab32351)

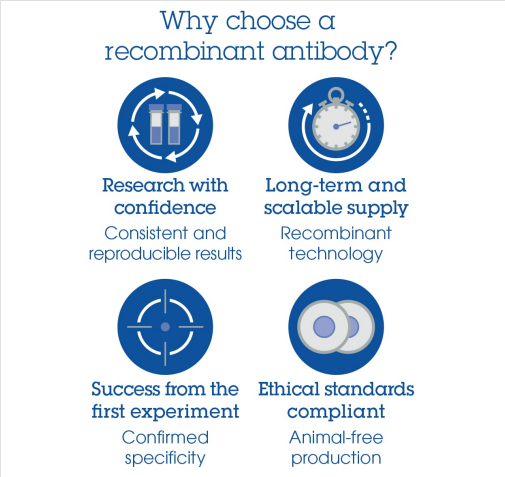
Carried out with unpurified antibody. Lane 1 = Caspase 3 protein (Active) (**ab52314**) 20 ng. Lane 2 = Caspase 9 protein (Active) (**ab52203**) 20 ng. Lane 3 = Extract of HeLa (human epithelial cell line from cervix adenocarcinoma) cells treated with vehicle (**ab136806**) 20 ug. Lane 4 = Extract of HeLa cells treated with staurosporine (**ab136806**) 20 ug. SDS PAGE performed under reducing conditions (100 mM DTT Sample heated at 50°C). Primary : Lanes 1-4: Anti Caspase 3 antibody (ab32351) at 1:1000 dilution. Secondary : Lanes 1-4: Goat anti rabbit IgG(H&L)-HRP at 1:10000. Development: ECL for 10 min exposure. Blocking: in 5% Milk + PBS overnight at 4 C. Primary antibody: in 5% Milk + PBS for 2 hours at RT. Secondary antibody: in 5% Milk + PBS for 2 hours at RT. Predicted band size : 32 kDa and 17 kDa. Observed band size : 32 kDa and 17 kDa.



Flow Cytometry (Intracellular) - Anti-Caspase-3 antibody [E87] (ab32351)

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with unpurfied ab32351 (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 followed by the antibody (ab32351, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor®488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (**ab172730**, 0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Anti-Caspase-3 antibody [E87] (ab32351)

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