abcam

Product datasheet

Anti-Caspase-3 antibody [E87] - BSA and Azide free ab197202

KO VALIDATED Recombinant RabMAb

51 References 9 Images

Overview

Product name	Anti-Caspase-3 antibody [E87] - BSA and Azide free
Description	Rabbit monoclonal [E87] to Caspase-3 - BSA and Azide free
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:** Flow Cyt (Intra), IP, IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 whole cell lysate. 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	ab197202 is the carrier-free version of **ab32351**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E87
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab197202 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
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 35 kDa (predicted molecular weight: 32 kDa).
ICC/IF		Use at an assay dependent concentration. **For unpurified, use 1/25 dilution.**

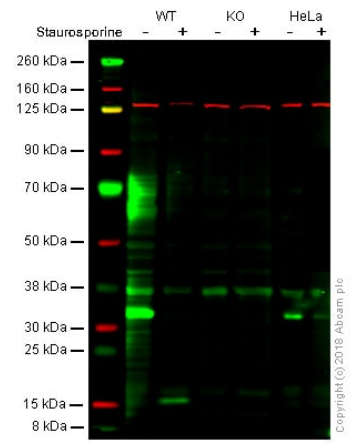
Target

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] - BSA and Azide free (ab197202)

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

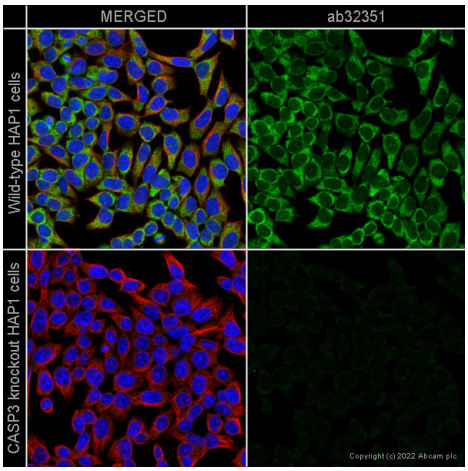
Lysates/proteins at 20 µg per lane.

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.

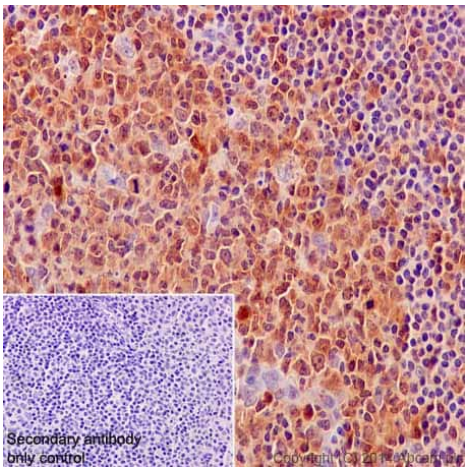
ab32351 was shown to recognize Caspase 3 in wild-type HAP1 cells as signal was lost at the expected MW in HAP1 Staurosporine Treated (CASP3) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and HAP1 Staurosporine Treated (CASP3) knockout samples 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32351**).



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-3 antibody [E87] - BSA and Azide free (ab197202)

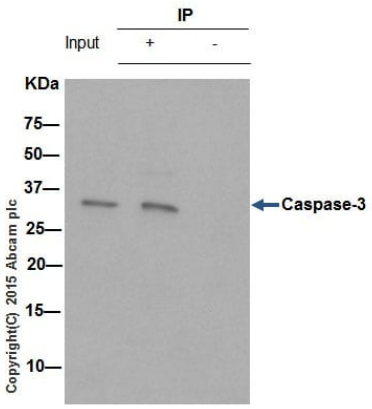
This data was developed using the same antibody clone in a different buffer formulation (**ab32351**). **ab32351** staining Caspase-3 in wild-type Hap1 cells (top panel) and CASP3 knockout Hap1 cells (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 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] - BSA and Azide free (ab197202)

This IHC data was generated using the same anti-Caspase 3 antibody clone, E87, in a different buffer formulation (cat# **ab32351**).

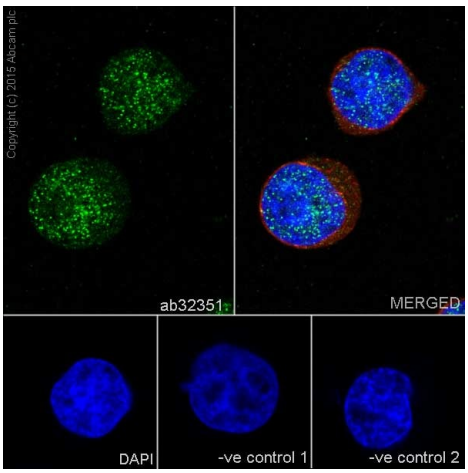
Immunohistochemical staining of paraffin embedded human tonsil with purified **ab32351** at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer,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



Immunoprecipitation - Anti-Caspase-3 antibody [E87] - BSA and Azide free (ab197202)

ab32351 (purified) at 1/50 immunoprecipitating Cullin 1 in 10 µg HeLa 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 at 1/1500 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST

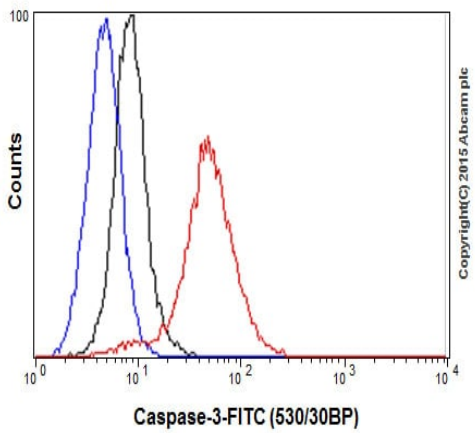
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32351**).



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-3 antibody [E87] - BSA and Azide free (ab197202)

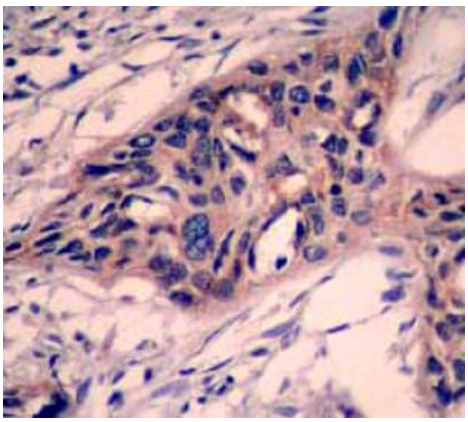
Immunofluorescence staining of Jurkat cells with purified **ab32351** at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32351**).



Flow Cytometry (Intracellular) - Anti-Caspase-3 antibody [E87] - BSA and Azide free (ab197202)

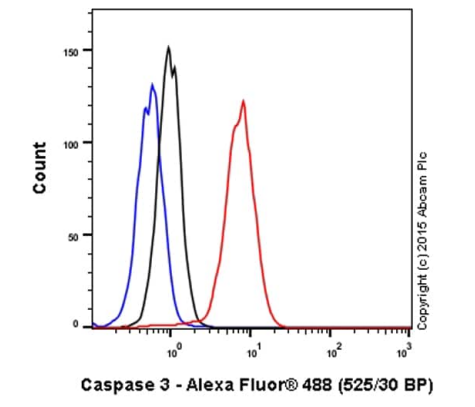
Overlay histogram showing Ramos cells fixed in 4% PFA and stained with purified **ab32351** at a dilution of 1 in 180 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32351**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [E87] - BSA and Azide free (ab197202)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32351**).



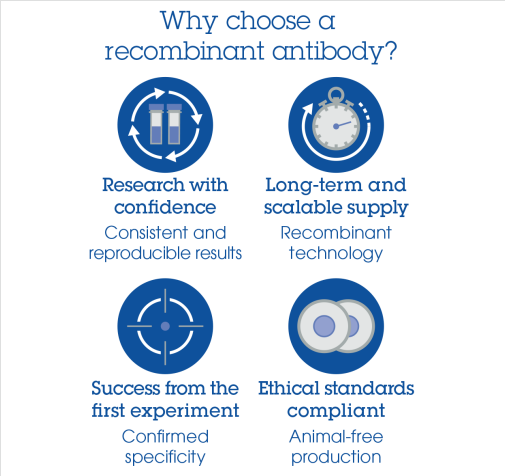
Flow Cytometry (Intracellular) - Anti-Caspase-3 antibody [E87] - BSA and Azide free (ab197202)

Overlay histogram showing HeLa 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 Fluorr® 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.

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32351**).



Anti-Caspase-3 antibody [E87] - BSA and Azide free (ab197202)

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