abcam

Product datasheet

Anti-Caspase-3 antibody [31A1067] ab13585

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Overview

Product name	Anti-Caspase-3 antibody [31A1067]
Description	Mouse monoclonal [31A1067] to Caspase-3
Host species	Mouse
Specificity	ab13585 recognizes an active form of Caspase 3 after apoptosis has been induced in wildtype cells and not Caspase 3 knockout cells
Tested applications	**Suitable for:** WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant full length protein corresponding to Human Caspase-3 aa 1-277. Database link: **P42574**
Positive control	Staurosporine-treated HeLa or Jurkat cell lysate.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.4 Preservative: 0.05% Sodium azide Constituents: 99% PBS, 0.05% BSA
Purity	Protein G purified
Clonality	Monoclonal
Clone number	31A1067

Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab13585 in the following tested applications.

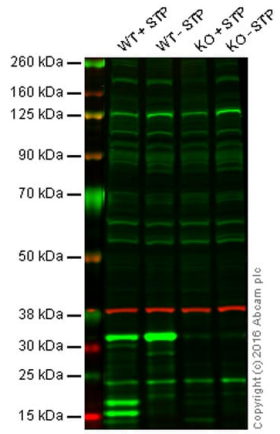
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use a concentration of 1 - 5 µg/ml. Predicted molecular weight: 31 kDa. The antibody detects both pro Caspase 3 (~32 kDa) and the large subunit of the active/cleaved form (~14-21 kDa) of Caspase 3. The large subunit of the cleaved form may appear as one or two or even as a stack of bands depending on the presence or absence of the Caspase 3 pro-domain.

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.

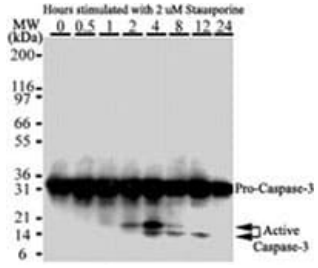
Images



Western blot - Anti-Caspase-3 antibody [31A1067] (ab13585)

Lane 1: Wild-type HAP1 cell lysate + Staurosporine (1µM for 4h)
Lane 2: Wild-type HAP1 cell lysate
Lane 3: Caspase-3 knockout HAP1 cell lysate + Staurosporine (1µM for 4h)
Lane 4: Caspase-3 knockout HAP1 cell lysate
Lanes 1 - 4: Merged signal (red and green). Green - ab13585 observed at 32 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

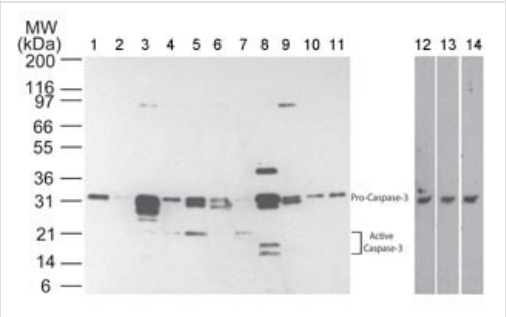
ab13585 was shown to recognise pro Caspase 3 when Caspase 3 knockout samples were used, along with additional cross-reactive bands. Wild-type and Caspase 3 knockout samples (± Staurosporine treatment) were subjected to SDS-PAGE. ab13585 at a concentration of 1 µg/ml and **ab8245** (loading control to GAPDH) diluted to 1/10000 were incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Caspase-3 antibody [31A1067] (ab13585)

Western blot analysis of Caspase 3 in HeLa cells using ab13585. Cells were treated with 2 uM staurosporine for different time periods. Caspase 3 activation is detected in Western blots by the presence of Caspase 3 cleavage fragments.

ab13585 detected both pro (full-length) and active (cleaved) Caspase 3, depending on the treatment time points. Pro Caspase 3 is detected at ~32 kDa. Active/cleaved Caspase 3 (large subunit) is detected at ~14-21 kDa as one or more bands.



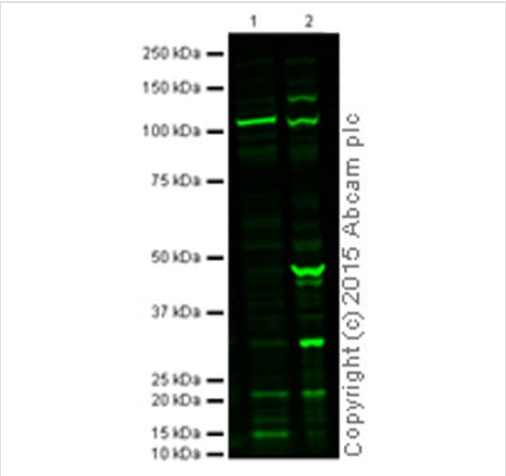
Western blot - Anti-Caspase-3 antibody [31A1067] (ab13585)

All lanes : Anti-Caspase-3 antibody [31A1067] (ab13585) at 5 µg/ml

Lane 1 : Human brain lysate
Lanes 2 & 12 : Human heart lysate
Lane 3 : Human intestine lysate
Lane 4 : Human kidney lysate
Lane 5 : Human liver lysate
Lane 6 : Human lung lysate
Lane 7 : Human muscle lysate
Lane 8 : Human stomach lysate
Lane 9 : Human spleen lysate
Lane 10 : Human ovary lysate
Lane 11 : Human testis lysate
Lane 13 : Mouse heart lysate
Lane 14 : Rat heart lysate

Predicted band size: 31 kDa

Lanes 12, 13 and 14 demonstrate the species cross-reactivity of the antibody in Human, mouse and rat heart lysate, respectively.



Western blot - Anti-Caspase-3 antibody [31A1067] (ab13585)

All lanes : Anti-MDC1 antibody (**ab13858**) at 1 µg/ml

Lane 1 : Hela whole cell lysate (Staurosporine treated, 2uM/4hr)
Lane 2 : Hela whole cell lysate (untreated control)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (**ab175781**) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 31 kDa
Additional bands at: 17 kDa (possible mature (processed) protein), 19 kDa (possible mature (processed) protein), 32 kDa (possible immature (unprocessed))

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab13585 overnight at 4°C. Antibody binding was detected using **ab175781** at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

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