abcam

Product datasheet

Anti-Caspase-3 antibody ab13847

KO VALIDATED

56 Abreviews 1118 References 2 Images

Overview

Product name	Anti-Caspase-3 antibody
Description	Rabbit polyclonal to Caspase-3
Host species	Rabbit
Specificity	Stimulation is required to allow detection of the 17kDa cleaved form of the protein. Please see images below for recommended treatment conditions and positive controls. ab13847 recognizes a cleaved form of Caspase 3 (~17 kDa) after apoptosis has been induced in wildtype cells and not Caspase 3 knockout cells. Some customers have used this antibody successfully in IHC-P however our latest tests were unsuccessful and therefore we can no longer guarantee this application. We would recommend **ab32351** and **ab184787** as an alternative product for this application.
Tested applications	**Suitable for:** WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Dog, Pig, Xenopus laevis, Drosophila melanogaster, Indian muntjac, Zebrafish, Rhesus monkey, Chinese hamster, Common marmoset, Schmidtea mediterranea, Salvelinus alpinus
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Hap-1 WT, Human Caspase 3 (active) Recombinant Protein ICC: HeLa cells treated with 1 mM staurosporine
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: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab13847 in the following tested applications.

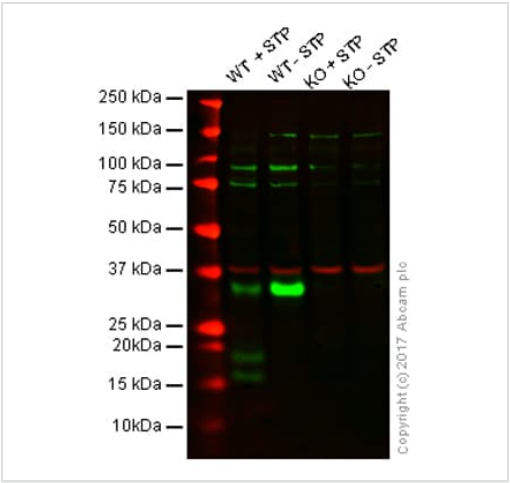
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ **(15)**	1/500. Detects a band of approximately 17, 34 kDa (predicted molecular weight: 17, 34 kDa).

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

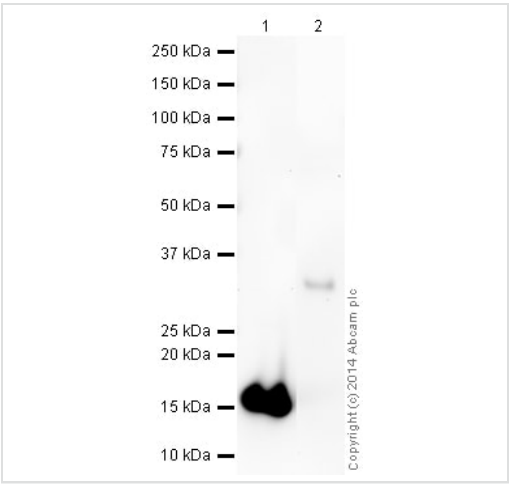


Western blot - Anti-Caspase-3 antibody (ab13847)

Lane 1: Wild-type HAP1 cell lysate + Staurosporine **(ab146588)** (1µM for 4h)
Lane 2: Wild-type HAP1 cell lysate
Lane 3: Caspase-3 knockout HAP1 cell lysate + Staurosporine (**ab146588**) (1µM for 4h)
Lane 4: Caspase-3 knockout HAP1 cell lysate

Lanes 1 - 4: Merged signal (red and green). Green - ab13847 observed at 17 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab13847 was shown to recognise 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. ab13847 and **ab8245** (loading control to GAPDH) were diluted to 1/500 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.



Western blot - Anti-Caspase-3 antibody (ab13847)

All lanes : Anti-Caspase-3 antibody (ab13847) at 1 µg/ml

Lane 1 : Human Caspase 3 (active) Recombinant Protein
Lane 2 : Human Pro Caspase 3 (inactive) Recombinant Protein

Lysates/proteins at 0.1 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 17, 34 kDa
Observed band size: 17,32 kDa

Exposure time: 8 minutes

Caspase 3 exist as inactive proenzymes which undergo proteolytic processing at conserved aspartic residues to produce large (17kDa) and small (12kDa) subunits. These subunits dimerize to form the active enzyme. ab13847 specifically detects the large active subunit (17kDa) and the inactive pro Caspase 3 (32 kDa).

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Bovine Serum Albumin before being incubated with ab13847 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.

Secondary antibody - **Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody**

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