

## Product datasheet

# Anti-Caspase-3 antibody ab13847

**KO** VALIDATED

★★★★☆ 42 Abreviews 254 References 9 Images

### Overview

|                            |   |
|----------------------------|---|
| <b>Product name</b>        | Anti-Caspase-3 antibody   |
| <b>Description</b>         | Rabbit polyclonal to Caspase-3  |
| <b>Host species</b>        | Rabbit  |
| <b>Specificity</b>         | ab13847 recognizes a cleaved form of Caspase 3 (~17 kDa) after apoptosis has been induced in wildtype cells and not Caspase 3 knockout cells.   |
| <b>Tested applications</b> | <b>Suitable for:</b> ICC/IF, IHC-P, IHC-Fr, WB, Flow Cyt, ICC, IHC (PFA fixed), IHC-FoFr, IHC - Wholemout   |
| <b>Species reactivity</b>  | <b>Reacts with:</b> Mouse, Rat, Human, Pig, Xenopus laevis, Drosophila melanogaster, Indian muntjac, Zebrafish, Rhesus monkey, Common marmoset, Schmidtea mediterranea, Salvelinus alpinus<br><b>Predicted to work with:</b> Dog, Chinese hamster |
| <b>Immunogen</b>           | Synthetic peptide corresponding to Human Caspase-3 aa 150-250 (internal sequence) conjugated to keyhole limpet haemocyanin.<br>Database link: <a href="#">P42574</a><br>(Peptide available as <a href="#">ab13848</a> )                           |
| <b>Positive control</b>    | IHC-P: Mouse embryo and liver tissue; Juvenile marmoset testis tissue. WB: HAP1 cell lysate; human caspase-3 recombinant protein. Flow Cyt: Jurkat cells. IHC-Fr: Tumour tissue; Rat brain tissue with a kainite lesion. ICC/IF: HeLa cells.      |

### Properties

|                             |  |
|-----------------------------|--|
| <b>Form</b>                 | Liquid   |
| <b>Storage instructions</b> | 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.   |
| <b>Storage buffer</b>       | pH: 7.40<br>Preservative: 0.02% Sodium azide<br>Constituent: PBS<br>Note: 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. |
| <b>Purity</b>               | Immunogen affinity purified  |

**Clonality** Polyclonal

**Isotype** IgG

## Applications

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   |
|------------------|-----------|---|
| ICC/IF           | ★★★★☆     | Use a concentration of 5 µg/ml.   |
| IHC-P            | ★★★★☆     | 1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.                               |
| IHC-Fr           | ★★★★★     | Use at an assay dependent concentration.  |
| WB               | ★★★★☆     | 1/500. Detects a band of approximately 17, 34 kDa (predicted molecular weight: 17, 34 kDa).                               |
| Flow Cyt         | ★★★★★     | 1/500.<br><a href="#">ab171870</a> - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody. |
| ICC              | ★★★★☆     | Use at an assay dependent concentration.  |
| IHC (PFA fixed)  |           | 1/300.  |
| IHC-FoFr         | ★★★★★     | 1/300.  |
| IHC - Wholemount | ★★★★☆     | 1/500.  |

## Target

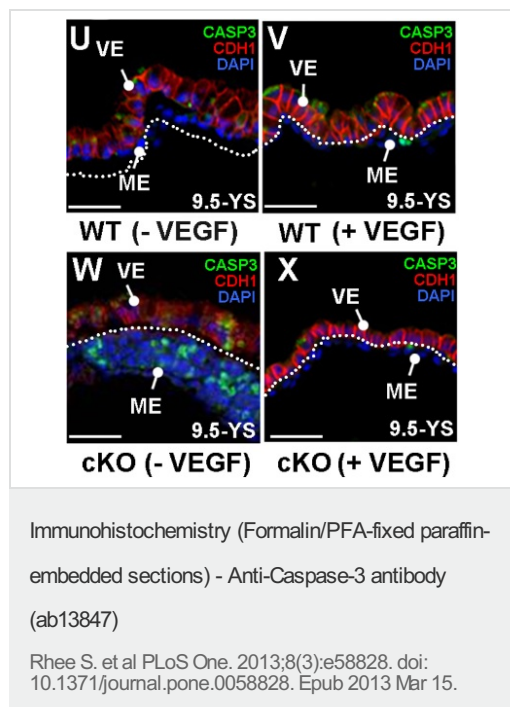
**Function** Involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis it proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp-Gly-217' bond. Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic 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.

## Images

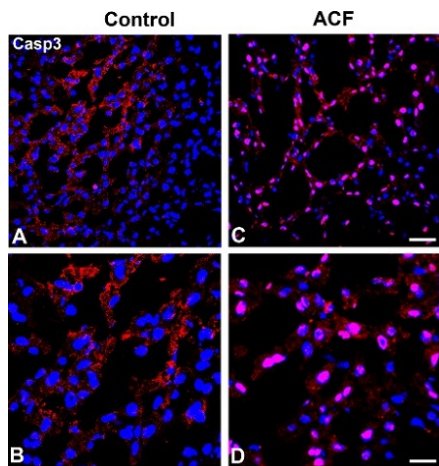


WT and cKO embryos cultured from 8.5–9.5 *dpc* in the presence (+VEGF) or absence (–VEGF) of VEGF.

Embryos and yolk sacs were dissected and fixed in 4% paraformaldehyde (PFA)/PBS overnight at 4°C. The following day they were washed in PBS, dehydrated in an ascending methanol sequence, xylene treated, embedded in paraffin and sectioned at 7.5  $\mu\text{m}$ .

For immunofluorescence (IF) slides were dewaxed in xylenes, rehydrated with EtOH, and subjected to antigen retrieval in Tris buffer pH 10.0 for 10 min. The slides were then washed in PBT and incubated in blocking buffer (0.5% milk powder, 99.5% PBT) for 2 hrs at room temperature and then with primary antibody (caspase-3, 1/500 dilution; CDH1, 1/500 dilution) in blocking buffer at 4°C overnight in a humid chamber. Slides were then washed three times with PBT, incubated for 1 hr with secondary antibody in blocking buffer at room temperature. Nuclei were counterstained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI, Molecular Probes, 1  $\times$  10,000) for 3 min and then coverslipped with Prolong Gold Antifade Reagent (Invitrogen). Sections were imaged on a Nikon Eclipse TE2000-S inverted microscope with Retiga EXi Fast camera with NIS Elements imaging software.

Immunofluorescence against cleaved Caspase-3 (CASP3, green) and CDH1 (red) of sectioned yolk sacs revealed that typical CDH1 expression found in WT (U) and WT cultured with VEGF (V), was downregulated in cultured cKO embryos but more normal visceral endoderm expression restored when cKO embryos were cultured with VEGF (X).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody (ab13847)

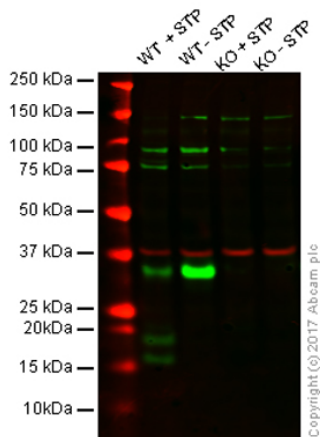
Shagura M et al PLoS One. 2017 Sep 21;12(9):e0184161. doi: 10.1371/journal.pone.0184161. eCollection 2017.

Confocal immunofluorescence microscopy of caspase 3 (red fluorescence) using an antibody detecting pro-caspase 3 recombinant protein (ab13847) with DAPI-counterstained nuclei (blue fluorescence) in liver sections of control or ACF adult rats.

Liver sections were incubated overnight with primary antibodies. After incubation with primary antibodies, the tissue sections were washed with PBS and then incubated with red fluorescent Alexa Fluor 594 donkey anti-rabbit antibody. Finally, the tissue sections were washed in PBS, mounted on vectashield (Vector Laboratories) and imaged on a confocal laser scanning microscope, LSM510, equipped with an argon laser (458/488/514 nm), a green helium/neon laser (543 nm), and a red helium/neon laser (633 nm; Carl Zeiss, Göttingen, Germany). Single optical slice images were taken using x10 or x20 Plan-Neofluar air interface or x40 Plan-Neofluar oil interface objective lens.

Caspase 3 immunoreactivity was confined primarily to the well defined subcellular organelle-like structures in hepatic cells of control rats (A, B). In contrast in ACF rats, caspase 3 immunofluorescence was transferred to the perinuclear area of cells or inside nuclei of hepatic cells indicating an activation of pro-apoptotic factor caspase 3 (C,D).

Bars = 20  $\mu$ m.



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

**Lane 1:** Wild-type HAP1 cell lysate + Staurosporine (ab146588) (1 $\mu$ M for 4h)

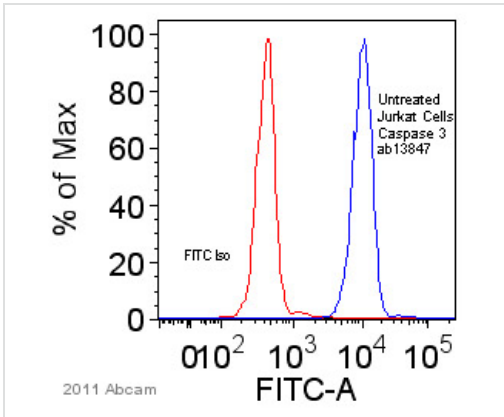
**Lane 2:** Wild-type HAP1 cell lysate

**Lane 3:** Caspase-3 knockout HAP1 cell lysate + Staurosporine (ab146588) (1 $\mu$ M 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 ( $\pm$ 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.

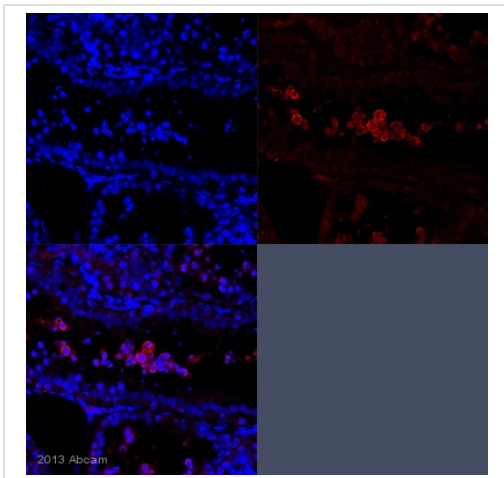


Flow Cytometry - Anti-Caspase-3 antibody (ab13847)

This image is courtesy of an anonymous Abreview

ab13847 staining active caspase 3 in Human Jurkat cells by Flow Cytometry. Cells were prepared in a phosphate buffered solution containing 0.1% sodium azide with FBS fixed with paraformaldehyde and permeabilized with Triton X-100 and NP40. The sample was incubated with the primary antibody (1/100 in wash buffer) for 24 hours at 4°C. A FITC-conjugated Goat anti-rabbit Ig (1/100) was used as the secondary antibody.

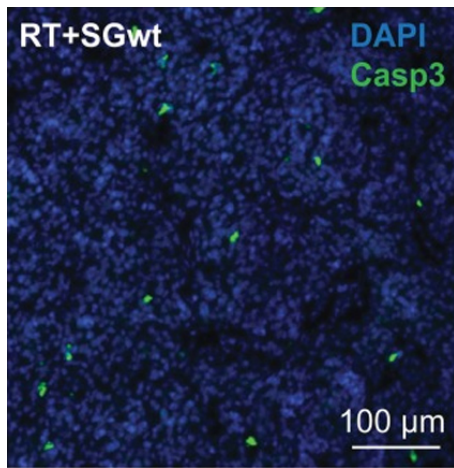
**Gating Strategy:** Isolate cell population from plot of SSC-A / FSA-A



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody (ab13847)

This image is courtesy of an abreview from Zachary Yu-Ching Lin

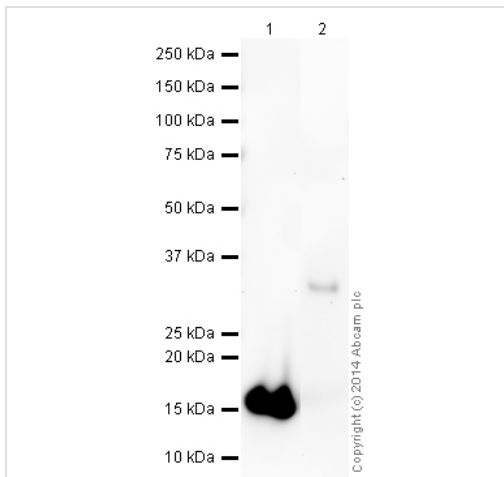
IHC-P image of Caspase 3 staining with ab13847 on tissue sections from juvenile marmoset testis. The sections were subjected to heat-mediated antigen retrieval using Dako antigen retrieval solution. The sections were then blocked with 5% milk for 30 minutes at 25°C, before incubation with ab13847 (1/100 dilution) for 18 hours at 4°C. The secondary was an Alexa-Fluor 555 conjugated goat anti-rabbit polyclonal, used at a 1/500 dilution.



Immunohistochemistry (Frozen sections) - Anti-Caspase-3 antibody (ab13847)

Image from PLoS One. 2015; 10(5): e0126688. Fig 2b, doi: 10.1371/journal.pone.0126688

5 μm frozen sections of tumor tissue were fixed with 100% ice cold methanol for 10 minutes, then blocked in 5% normal goat serum in PBS (pH 7.4) for 1h. Sections were incubated with ab13847 (1:500) at 4°C overnight and for 1h with secondary antibodies at room temperature.



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 [why is the actual band size different from the predicted?](#)

**Exposure time:** 8 minutes

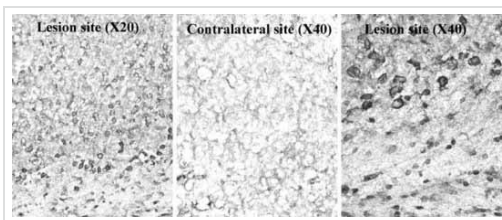
Caspase 3 exist as inactive proenzymes which undergo proteolytic processing at conserved aspartic residues to produce large (17kDa) and small (12kDa) subunits. These subunits dimerize to form the active enzyme. ab13847 specifically detects the large active subunit (17kDa) and the inactive pro Caspase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

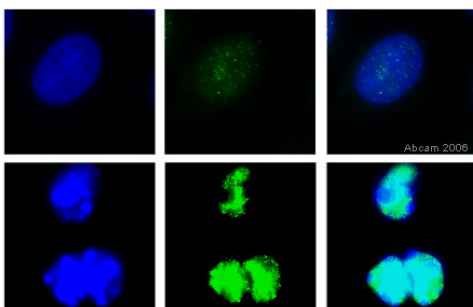
Secondary antibody - [Goat Anti-Rabbit IgG H&L \(HRP\) \(ab97051\)](#)  
secondary antibody



Immunohistochemistry (Frozen sections) - Anti-Caspase-3 antibody (ab13847)

This image is courtesy of an abreview submitted by Sophie Pezet, ESPCI, France

ab13847 was used in IHC of frozen sections from a rat brain with a kainite lesion. The non lesioned contralateral site serves as a negative control. The sections were fixed with paraformaldehyde. The tissue was perfused with 4% PFA and embedded in OCT compound and cut on the cryostat. The primary antibody was incubated for 12 hours at a dilution of 1/300. A biotin labelled secondary antibody was used at a dilution of 1/300.



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-3 antibody (ab13847)

This image is courtesy of Roberto Giambruno, Marilena Ciciarello and Patrizia Lavia

HeLa cells were fixed for 10 minutes at room temperature in 3.7% PFA and permeabilised in 0.1% Triton X-100/PBS then incubated with ab13847 (5µg/ml) for 1 hour at room temperature. The top panel shows control cells treated with DMSO. The bottom panel shows HeLa cells treated with 1 mM staurosporine ([ab146588](#)) for 4 hours to induce caspase-3 activation. ab13847 staining is shown in green and counterstaining with DAPI is shown in blue. 100x magnification.

The image shows the staining with ab13847 is very faint in the untreated control cultures, but very bright after activation of capsase-3 by treatment with the staurosporine . (N.B. in these cultures the nuclei are apoptotic).

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