abcam

Product datasheet

Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free ab224271

KO VALIDATED Recombinant RabMAb

1 References 7 Images

Overview

Product name	Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free
Description	Rabbit monoclonal [EPR18297] to Caspase-3 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody recognizes pro-Caspase 3 and potentially cross reacts with active caspases after apoptosis has been induced in wildtype cells and not Caspase 3 knockout cells
Tested applications	**Suitable for:** IP, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 treated Staurosporine (2 uM, 4h) and Vehicle Control Staurosporine (0 uM, 4h), Wild-type HeLa treated Staurosporine (2 uM, 4h) and Vehicle Control Staurosporine (0 uM, 4h), untreated NIH/3T3 and treated with 1µM Staurosporine for 4 hours, untreated Jurkat and treated with 1uM Staurosporine for 4 hours, mouse brain, mouse and rat brain lysates. Human brain, heart and liver tissue. IHC-P: Human tonsil and cervical cancer tissues. IP: HeLa lysate treated with 1uM Staurosporine for 4 hours.
General notes	ab224271 is the carrier-free version of **ab184787**. 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. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18297
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab224271 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
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 32, 17 kDa (predicted molecular weight: 32 kDa). The 17 kDa band is the active form of the cleaved caspase 3 (subunit p17).
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.

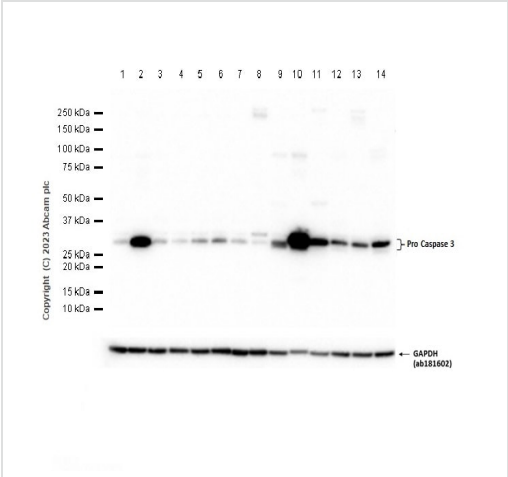
Target

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 [EPR18297] - BSA and Azide free (ab224271)

All lanes : Anti-Caspase-3 antibody [EPR18297] (**ab184787**) at 1/1000 dilution

Lane 1 : Mouse Alzheimer's disease brain tissue lysate
Lane 2 : Mouse brain cancer tissue lysate
Lane 3 : Mouse hippocampus tissue lysate
Lane 4 : Mouse spinal cord tissue lysate
Lane 5 : Mouse cerebellum tissue lysate
Lane 6 : Mouse cerebral cortex tissue lysate
Lane 7 : Mouse hypothalamus tissue lysate
Lane 8 : Mouse heart tissue lysate
Lane 9 : Mouse liver tissue lysate
Lane 10 : Human brain tissue lysate
Lane 11 : Human liver tissue lysate
Lane 12 : Human hypothalamus tissue lysate
Lane 13 : Human heart tissue lysate
Lane 14 : Human cerebellum tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 32 kDa
Observed band size: 27-32 kDa

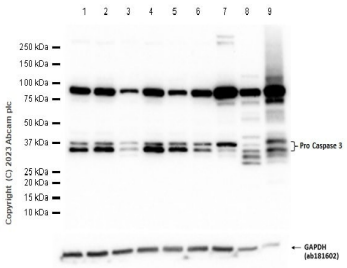
Exposure time: 10 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184787**).

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

Bands between 27-32kDa represent cleavage of the procaspase at D9 and D28, respectively (PMID: 14567691)



Western blot - Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free (ab224271)

All lanes : Anti-Caspase-3 antibody [EPR18297] (**ab184787**) at 1/1000 dilution

Lane 1 : Rat brain tissue lysate
Lane 2 : Rat hippocampus tissue lysate
Lane 3 : Rat spinal cord tissue lysate
Lane 4 : Rat cerebellum tissue lysate
Lane 5 : Rat cerebral cortex tissue lysate
Lane 6 : Rat hypothalamus tissue lysate
Lane 7 : Rat heart tissue lysate
Lane 8 : Rat liver tissue lysate
Lane 9 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 32 kDa
Observed band size: 27-32 kDa

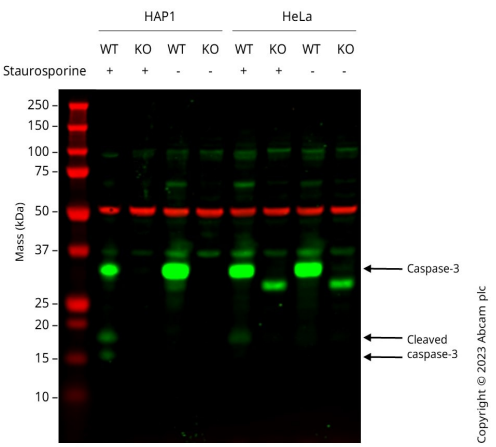
Exposure time: 20 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184787**).

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

Bands between 27-32kDa represent cleavage of the procaspase at D9 and D28, respectively (PMID: 14567691)



Western blot - Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free (ab224271)

All lanes : Anti-Caspase-3 antibody [EPR18297] (**ab184787**) at 1/2000 dilution

Lane 1 : Wild-type HAP1 Treated Staurosporine (2 uM, 4h) cell lysate
Lane 2 : CASP3 knockout HAP1 Treated Staurosporine (2 uM, 4h) cell lysate
Lane 3 : Wild-type HAP1 Vehicle Control Staurosporine (0 uM, 4h) cell lysate
Lane 4 : CASP3 knockout HAP1 Vehicle Control Staurosporine (0 uM, 4h) cell lysate
Lane 5 : Wild-type HeLa Treated Staurosporine (2 uM, 4h) cell lysate
Lane 6 : CASP3 knockout HeLa Treated Staurosporine (2 uM, 4h) cell lysate
Lane 7 : Wild-type HeLa Vehicle Control Staurosporine (0 uM, 4h) cell lysate
Lane 8 : CASP3 knockout HeLa Vehicle Control Staurosporine (0 uM, 4h) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

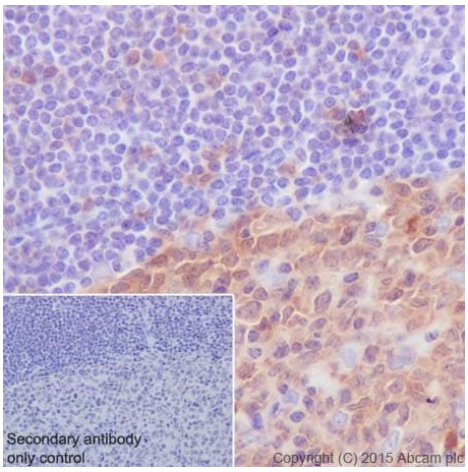
Performed under reducing conditions.

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

Anti-CASP3 antibody [EPR18297] (**ab184787**) staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab184787** was shown to bind specifically to CASP3. A band was observed at 35 kDa in treated wild-type HAP1 and HeLa cell lysates with no signal observed at this size in CASP3 knockout HAP1 cell line and a band at a lower molecular weight in the CAPS3 knockout HeLa cell line **ab255370** (knockout cell lysate **ab263779**) which cannot be cleaved to active CASP3. To generate this image, wild-type and CASP3 knockout HAP1 cell lysates were

analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184787**).



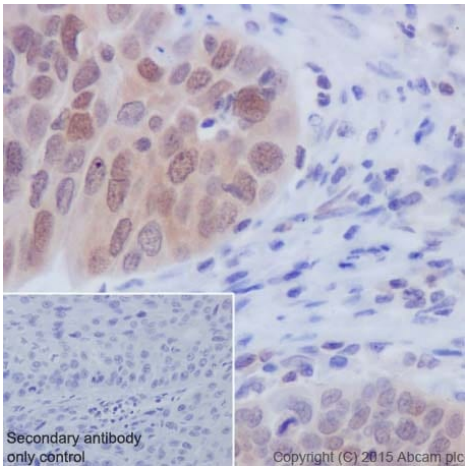
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free (ab224271)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling active and pro Caspase 3 with **ab184787** at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nucleus and cytoplasm staining on lymphocytes of tonsil is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184787**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



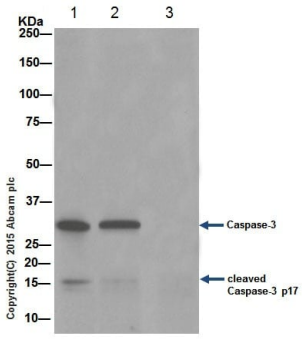
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free (ab224271)

Immunohistochemical analysis of paraffin-embedded Human cervical cancer tissue labeling active and pro Caspase 3 with **ab184787** at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nucleus and cytoplasm staining on tumor cells of Human cervix cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184787**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free (ab224271)

active and pro Caspase 3 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate treated with 1uM staurosporine for 4 hours with **ab184787** at 1/80 dilution. Western blot was performed from the immunoprecipitate using **ab184787** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/1500 dilution.

Lane 1: HeLa whole cell lysate treated with 1uM staurosporine for 4 hours 10 µg (Input).

Lane 2: **ab184787** IP in HeLa whole cell lysate treated with 1uM staurosporine for 4 hours.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab184787** in HeLa whole cell lysate treated with 1uM staurosporine for 4 hours.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab184787**).

Anti-Caspase-3 antibody [EPR18297] - BSA and Azide free (ab224271)

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