abcam

Product datasheet

Anti-Caspase-3 antibody [EPR18297] ab184787

敲除验证 重组 RabMAb

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概述

产品名称	Anti-Caspase-3抗体[EPR18297]
描述	兔单克隆抗体[EPR18297] to Caspase-3
宿主	Rabbit
特异性	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
经测试应用	**适用于:** WB, IHC-P, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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 and rat brain tissue 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.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR18297
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab184787于以下的经测试应用

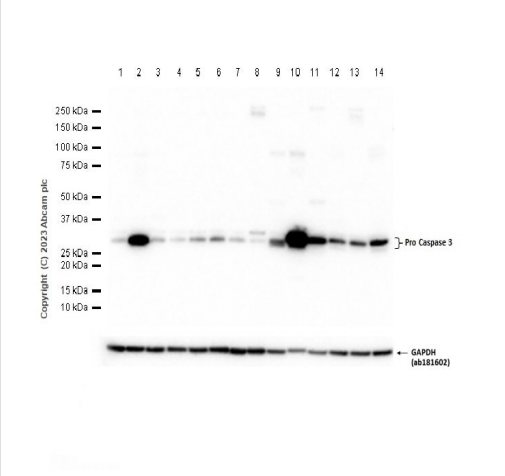
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★☆☆ (2)	1/2000. 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). ab184787 recognizes pro-Caspase 3 and unable to detect the active caspases after induction in mouse and rat samples.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/80.

靶标

功能	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.
组织特异性	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.
序列相似性	Belongs to the peptidase C14A family.
翻译后修饰	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.
细胞定位	Cytoplasm.

图片



Western blot - Anti-Caspase-3 antibody [EPR18297] (ab184787)

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

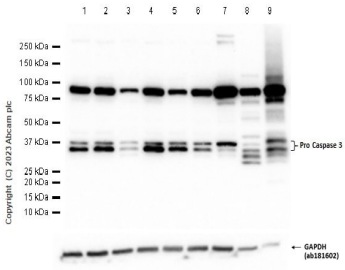
Predicted band size: 32 kDa
Observed band size: 27-32 kDa

Exposure time: 10 seconds

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] (ab184787)

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

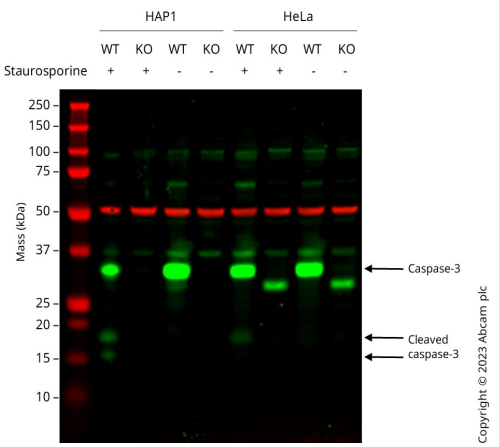
Predicted band size: 32 kDa
Observed band size: 27-32 kDa

Exposure time: 20 seconds

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] (ab184787)

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.

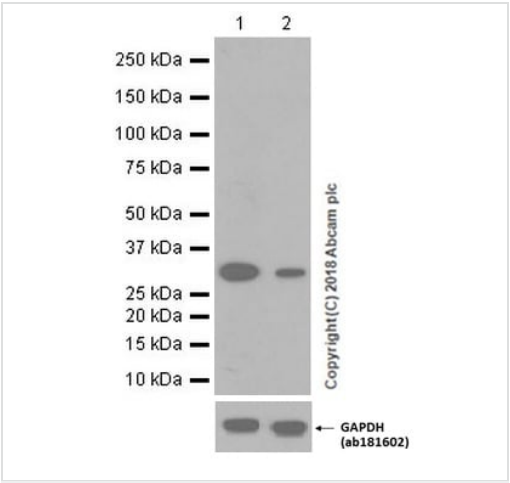
Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

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.



Western blot - Anti-Caspase-3 antibody [EPR18297] (ab184787)

All lanes : Anti-Caspase-3 antibody [EPR18297] (ab184787) at 0.7 µg/ml

Lane 1 : Untreated NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 1µM Staurosporine for 4 hours whole cell lysate

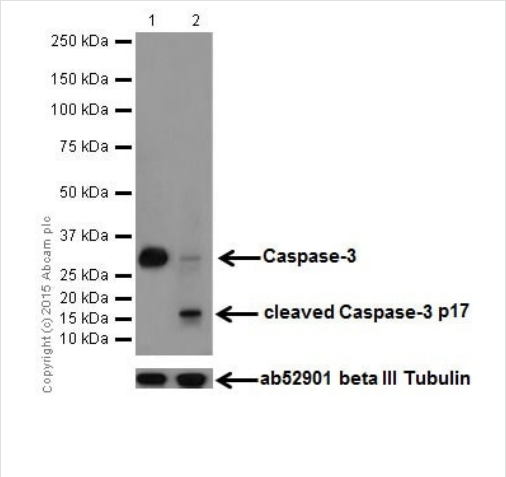
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Blocking and diluting buffer: 5% NFDM/TBST.

ab184787 recognizes pro-Caspase 3 and unable to detect the active caspases after induction in mouse and rat samples.



Western blot - Anti-Caspase-3 antibody [EPR18297] (ab184787)

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

Lane 1 : Untreated Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates
Lane 2 : Jurkat whole cell lysates treated with 1uM staurosporine for 4 hours

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

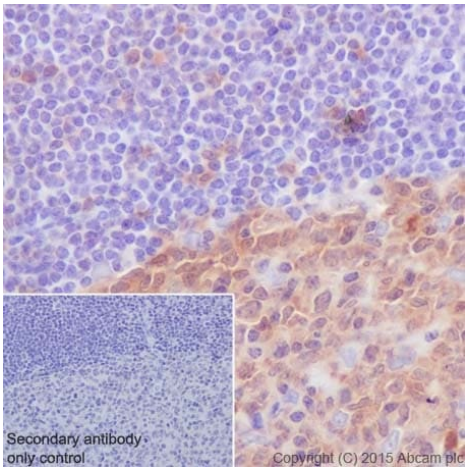
Predicted band size: 32 kDa
Observed band size: 17,32 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

Specificity: interacts with full length pro-Caspase 3 and the p17 subunit.

The Caspase-3 precursor is first cleaved between D175 and S176 to produce the p11 subunit and p20 fragment. Subsequently, the p20 fragment is cleaved between D28 and S29 to generate the p17 subunit (Proc. Natl. Acad. Sci. USA. 93, 7464-7469 - PMID:8755496).

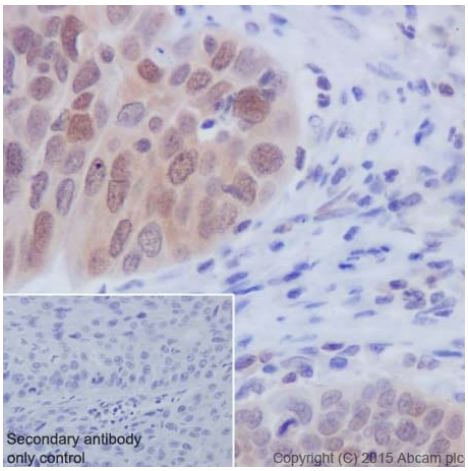


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [EPR18297] (ab184787)

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.

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

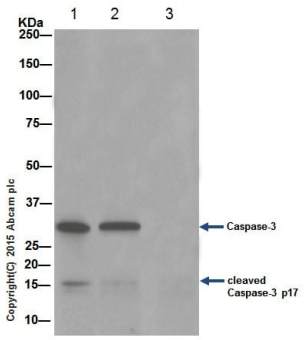


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [EPR18297] (ab184787)

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.

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



Immunoprecipitation - Anti-Caspase-3 antibody [EPR18297] (ab184787)

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.

Anti-Caspase-3 antibody [EPR18297] (ab184787)

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