abcam

Product datasheet

Anti-Caspase-9 antibody [EPR18107] ab202068

敲除验证 重组 RabMAb

4 Abreviews 97 References 11 图像

概述

产品名称	Anti-Caspase-9抗体[EPR18107]
描述	兔单克隆抗体[EPR18107] to Caspase-9
宿主	Rabbit
经测试应用	**适用于:** IHC-P, WB, ICC/IF, IP
种属反应性	**与反应:** Mouse, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa and C2C12 whole cell lysates; Human fetal brain, fetal heart, fetal kidney and fetal liver lysates. IHC-P: Human cervix carcinoma tissue. ICC/IF: HeLa cells. IP: HeLa treated with staurosporine 1uM for 4 hours whole cell lysate.
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, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR18107

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab202068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
IHC-P		1/300. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (4)	1/2000. Detects a band of approximately 46, 35, 37 kDa (predicted molecular weight: 46 kDa).
ICC/IF		1/500.
IP		1/80.

靶标

功能 Involved in the activation cascade of caspases responsible for apoptosis execution. Binding of caspase-9 to Apaf-1 leads to activation of the protease which then cleaves and activates caspase-3. Proteolytically cleaves poly(ADP-ribose) polymerase (PARP).
Isoform 2 lacks activity is an dominant-negative inhibitor of caspase-9.

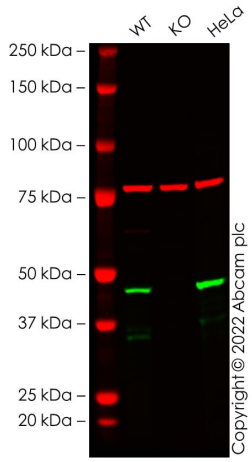
组织特异性 Ubiquitous, with highest expression in the heart, moderate expression in liver, skeletal muscle, and pancreas. Low levels in all other tissues. Within the heart, specifically expressed in myocytes.

序列相似性 Belongs to the peptidase C14A family.
Contains 1 CARD domain.

发展阶段 Expressed at low levels in fetal heart, at moderate levels in neonate heart, and at high levels in adult heart.

翻译后修饰 Cleavages at Asp-315 by granzyme B and at Asp-330 by caspase-3 generate the two active subunits. Caspase-8 and -10 can also be involved in these processing events.

图片



Western blot - Anti-Caspase-9 antibody [EPR18107] (ab202068)

All lanes : Anti-Caspase-9 antibody [EPR18107] (ab202068) at 1/2000 dilution

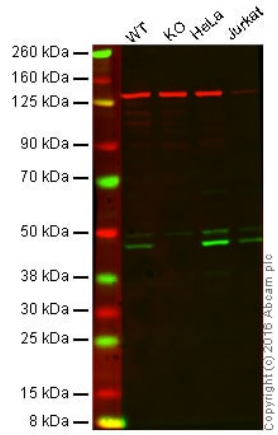
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : CASP9 knockout THP-1 cell lysate
Lane 3 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

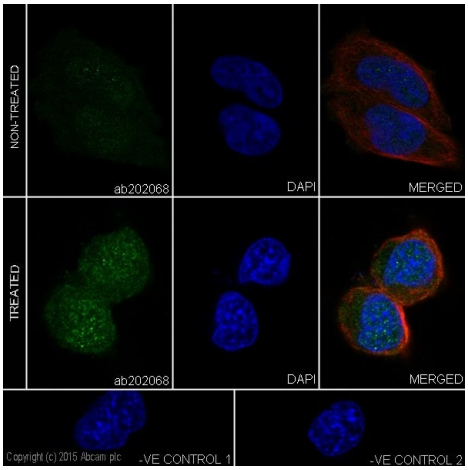
Predicted band size: 46 kDa
Observed band size: 45 kDa

False colour image of Western blot: Anti-Caspase-9 antibody [EPR18107] staining at 1/2000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab202068 was shown to bind specifically to Caspase-9. A band was observed at 45 kDa in wild-type THP-1 cell lysates with no signal observed at this size in CASP9 knockout cell line **ab276122** (knockout cell lysate **ab284219**). To generate this image, wild-type and CASP9 knockout THP-1 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-Caspase-9 antibody [EPR18107] (ab202068)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: Caspase-9 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Jurkat cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab202068 observed at 46 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab202068 was shown to recognize Caspase-9 when Caspase-9 knockout samples were used, along with additional cross-reactive bands. Wild-type and Caspase-9 knockout samples were subjected to SDS-PAGE. ab202068 and **ab8245** (loading control to GAPDH) were diluted at 1/2000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed withGoat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 h at room temperature before imaging.



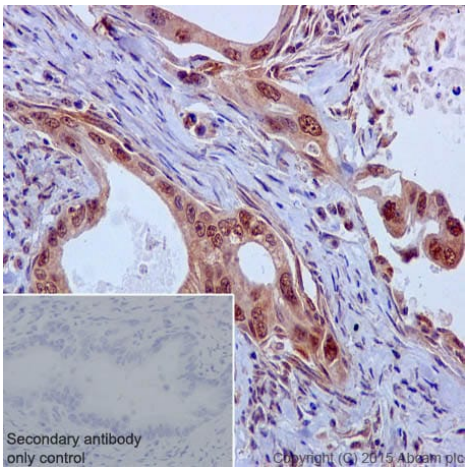
Immunocytochemistry/ Immunofluorescence - Anti-Caspase-9 antibody [EPR18107] (ab202068)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Caspase-9 with ab202068 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green).

Confocal image showing cytoplasmic and nuclear staining on HeLa cell line. The expression increased after treatment with staurosporine (1uM) for 4 hours.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
-ve control 1: **ab202067** at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-9 antibody [EPR18107] (ab202068)

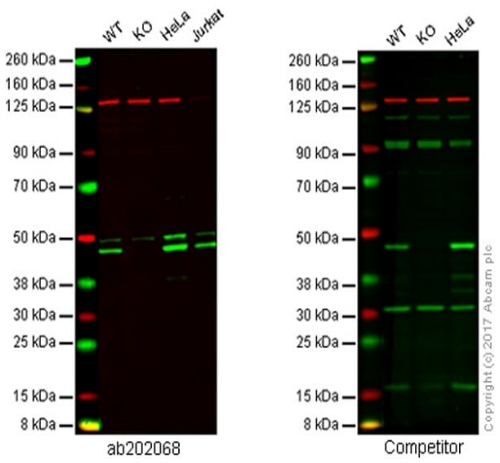
Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling Caspase-9 with ab202068 at 1/300 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution.

Cytoplasmic and nuclear staining on Human cervix carcinoma tissue 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.

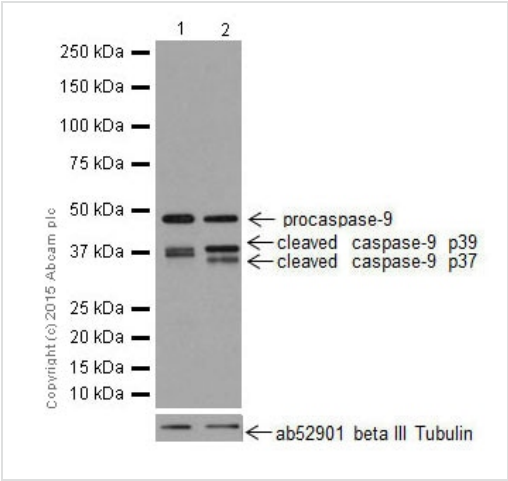


Western blot - Anti-Caspase-9 antibody [EPR18107] (ab202068)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: Caspase-9 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Jurkat cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green).

Green - target observed at 46 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab202068 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-Caspase-9 antibody [EPR18107] (ab202068)

All lanes : Anti-Caspase-9 antibody [EPR18107] (ab202068) at 1/10000 dilution

Lane 1 : Untreated C2C12 (Mouse myoblast cell line) whole cell lysate
Lane 2 : C2C12 (Mouse myoblast cell line) treated with staurosporine 1uM for 4 hours whole cell lysate

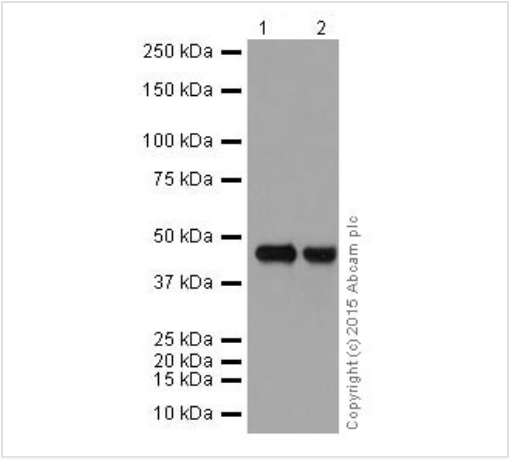
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 46 kDa
Observed band size: 37,39,46 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Caspase-9 antibody [EPR18107] (ab202068)

All lanes : Anti-Caspase-9 antibody [EPR18107] (ab202068) at 1/2000 dilution

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal heart lysate

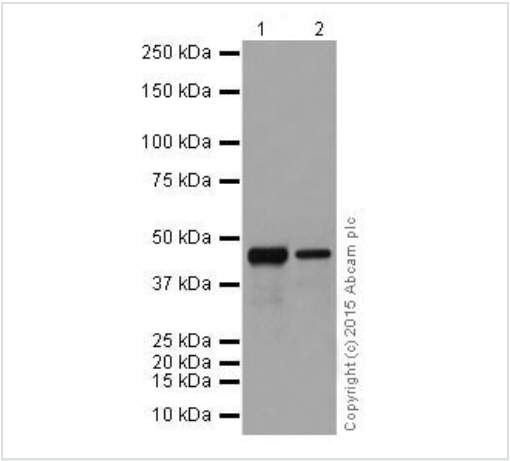
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 46 kDa
Observed band size: 46 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Caspase-9 antibody [EPR18107] (ab202068)

All lanes : Anti-Caspase-9 antibody [EPR18107] (ab202068) at 1/2000 dilution

Lane 1 : Human fetal kidney lysate
Lane 2 : Human fetal liver lysate

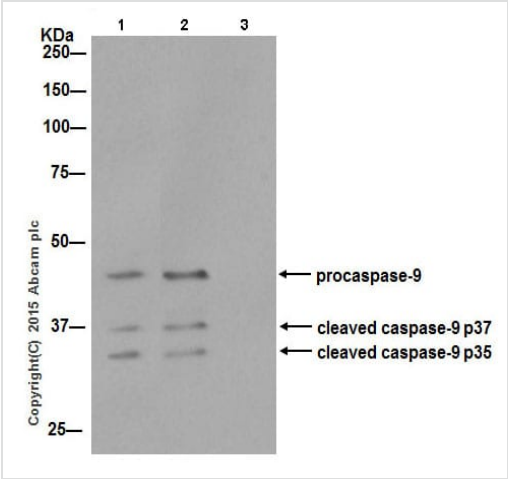
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 46 kDa
Observed band size: 46 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-Caspase-9 antibody [EPR18107] (ab202068)

Caspase-9 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) treated with staurosporine 1uM for 4 hours whole cell lysate with ab202068 at 1/80 dilution.

Western blot was performed from the immunoprecipitate using ab202068 at 1/1000 dilution.

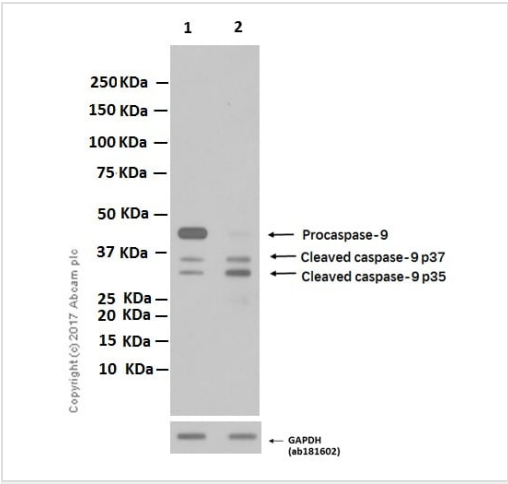
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG was used as secondary antibody at 1/1500 dilution.

Lane 1: HeLa treated with staurosporine 1uM for 4 hours whole cell lysate10 µg (Input).

Lane 2: ab202068 IP in HeLa treated with staurosporine 1uM for 4 hours whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab202068 in HeLa treated with staurosporine 1uM for 4 hours whole cell lysate. Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.



Western blot - Anti-Caspase-9 antibody [EPR18107] (ab202068)

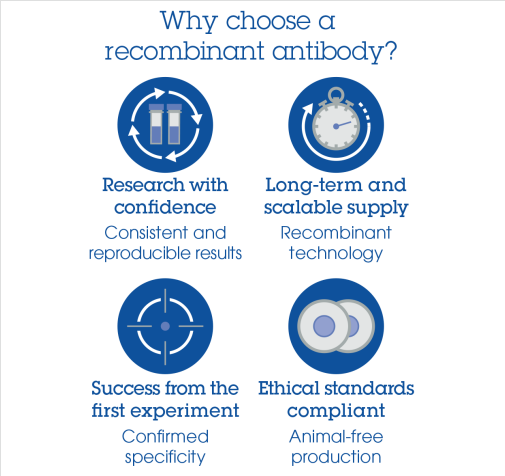
All lanes : Anti-Caspase-9 antibody [EPR18107] (ab202068) at 1/50000 dilution

Lane 1 : Untreated HeLa (human epithelial cells from cervix adenocarcinoma) whole cell lysate at 10 µg
Lane 2 : HeLa (human epithelial cells from cervix adenocarcinoma) treated with staurosporine 1 µM for 4 hours whole cell lysate at 20 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 46 kDa
Observed band size: 35,37,46 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-Caspase-9 antibody [EPR18107] (ab202068)

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