

Anti-Caspase-9 antibody [E23] ab32539

敲除验证
重组
RabMAb

★★★★☆
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概述

产品名称	Anti-Caspase-9抗体[E23]
描述	兔单克隆抗体[E23] to Caspase-9
宿主	Rabbit
特异性	This antibody should recognise both the pro-[40kDa] form and p35 cleaved form of Caspase-9.
经测试应用	适用于: WB, IHC-P, IP, Flow Cyt (Intra), ICC/IF
种属反应性	与反应: Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Jurkat and HeLa whole cell lysate (ab150035). IHC-P: Human skeletal muscle and cervical carcinoma tissues. ICC/IF: HepG2 cells. Flow Cyt (intra): K562 cells. IP: HeLa whole cell lysate (ab150035).
常规说明	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

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. Avoid freeze / thaw cycle.
存储溶液	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol, 0.05% BSA</p>

纯度	Protein A purified
克隆	单克隆
克隆编号	E23
同种型	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab32539于以下的经测试应用

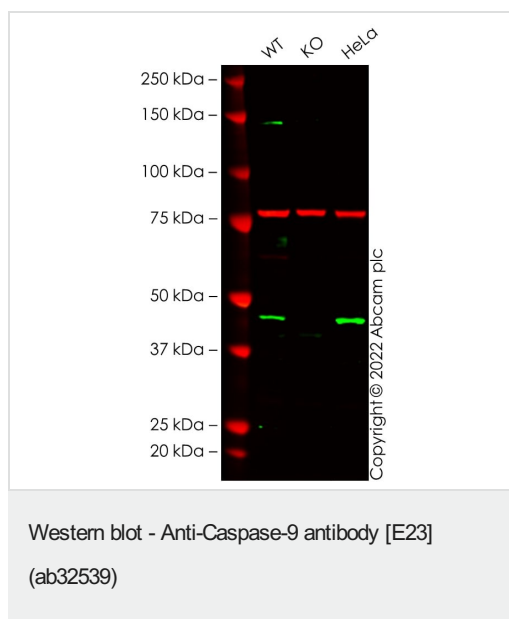
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (4)	1/1000 - 1/10000. Predicted molecular weight: 46 kDa. We recommend overnight incubation at 4°C.
IHC-P		1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See <u>IHC antigen retrieval protocols</u> .
IP		1/80 - 1/100.
Flow Cyt (Intra)		1/100 - 1/250. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500. For unpurified use at 1/50.

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



All lanes : Anti-Caspase-9 antibody [E23] (ab32539) at 1/1000 dilution

Lane 1 : Wild-type THP-1 cell lysate

Lane 2 : CASP9 knockout THP-1 cell lysate

Lane 3 : HeLa cell lysate

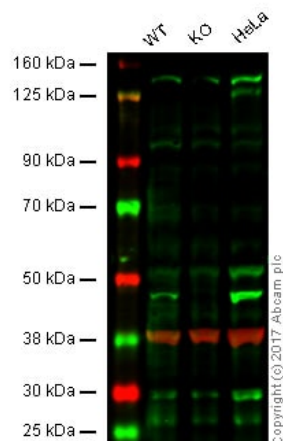
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 46 kDa

Observed band size: 45 kDa

False colour image of Western blot: Anti-Caspase-9 antibody [E23] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] ([ab238078](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32539 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 [E23]
(ab32539)

Lanes:

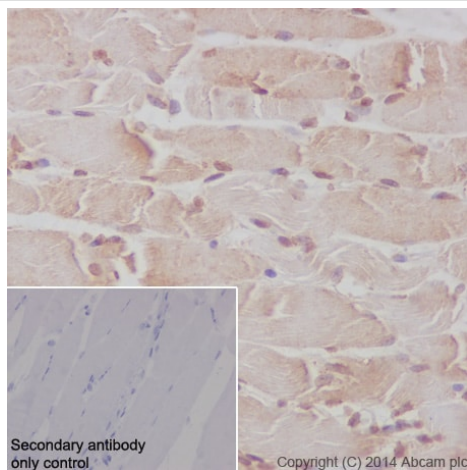
Lane 1: Wild-type HAP1 whole cell lysate (40 µg)

Lane 2: CASP9 knockout HAP1 whole cell lysate (40 µg)

Lane 3: HeLa whole cell lysate (40 µg)

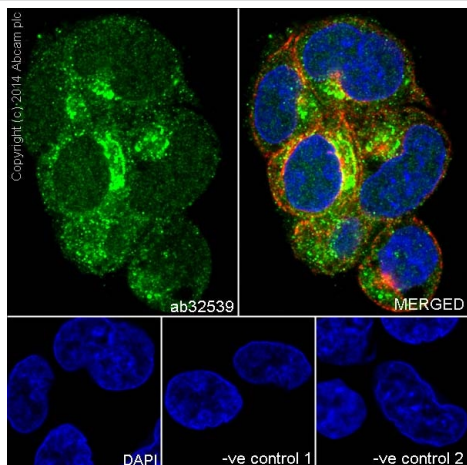
Lanes 1 - 4: Merged signal (red and green). Green - ab32539 observed at 45 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32539 was shown to specifically recognize Caspase-9 in wild type HAP1 cells along with additional cross-reactive bands. No band was observed when Caspase-9 knockout samples were examined. Wild-type and Caspase-9 knockout samples were subjected to SDS-PAGE. Ab32539 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

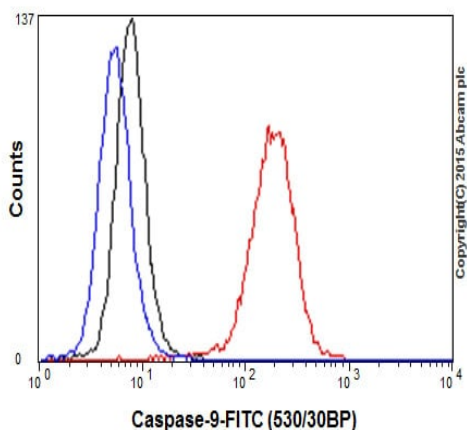


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-9 antibody [E23] (ab32539)

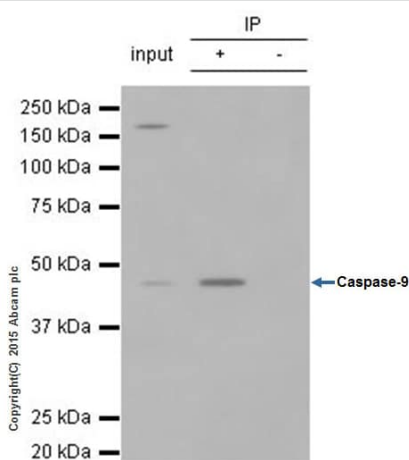
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle tissue labelling Caspase-9 with purified ab32539 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-9 antibody [E23] (ab32539)



Flow Cytometry (Intracellular) - Anti-Caspase-9 antibody [E23] (ab32539)



Immunoprecipitation - Anti-Caspase-9 antibody [E23] (ab32539)

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling Caspase-9 with purified ab32539 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/500) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).

Intracellular Flow Cytometry analysis of K562 cells labelling Caspase-9 with purified ab32539 at 1/250 (red). Cells were fixed with 100% methanol. A FITC-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

ab32539 (purified) at 1/80 immunoprecipitating Caspase-9 in HeLa whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10µg)

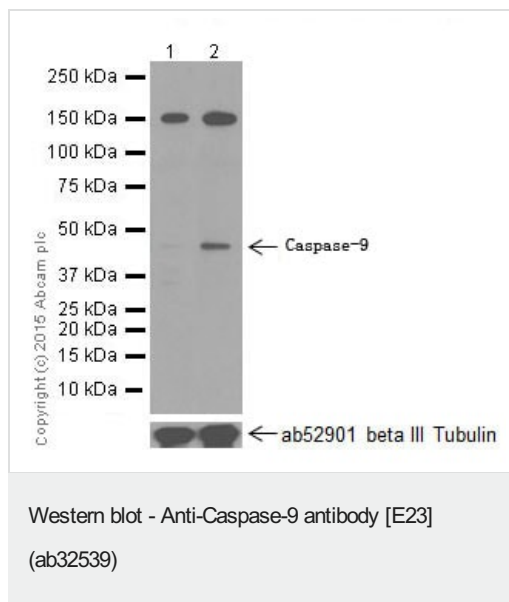
Lane 2 (+): ab32539 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32539 in HeLa whole cell lysate.

For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



All lanes : Anti-Caspase-9 antibody [E23] (ab32539) at 1/1000 dilution (purified)

Lane 1 : HeLa whole cell lysate - treated with Camptothecin

Lane 2 : HeLa whole cell lysate - untreated

Lysates/proteins at 10 µg per lane.

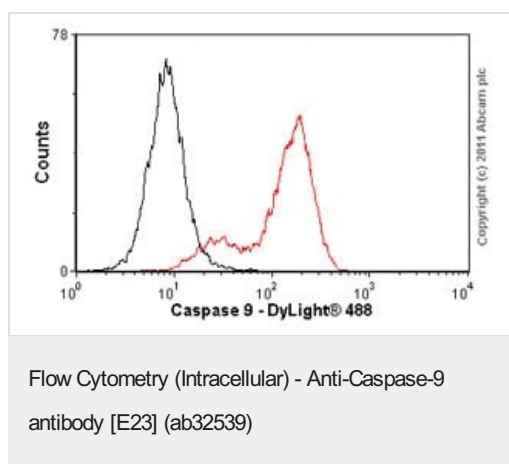
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

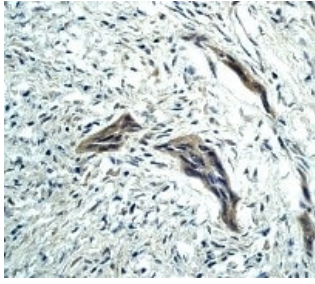
Predicted band size: 46 kDa

Observed band size: 35,46 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Overlay histogram showing K562 cells stained with unpurified ab32539 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab32539, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was anti-rabbit DyLight® 488 (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in K562 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-9 antibody [E23] (ab32539)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling Caspase 9 with unpurified ab32539 at a dilution of 1/50.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-Caspase-9 antibody [E23] (ab32539)

All lanes : Anti-Caspase-9 antibody [E23] (ab32539) at 1/20000 dilution (unpurified)

Lane 1 : Jurkat cell lysate - untreated

Lane 2 : Jurkat cell lysate - treated with Camptothecin

Predicted band size: 46 kDa

Observed band size: 48 kDa

Additional bands at: 28 kDa. We are unsure as to the identity of these extra bands.

Why choose a recombinant antibody?



Anti-Caspase-9 antibody [E23] (ab32539)

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