abcam

Product datasheet

Anti-Caspase-9 antibody [E84] ab32068





15 References 5 Images

Overview

Product name Anti-Caspase-9 antibody [E84]

Description Rabbit monoclonal [E84] to Caspase-9

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Unsuitable for: ICC/IF or IP

Reacts with: Human Species reactivity

Synthetic peptide within Human Caspase-9 aa 1-100 (N terminal). The exact sequence is **Immunogen**

proprietary.

Positive control Jurkat cell lysate

General notes 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.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

Form

Storage instructions Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number E84
Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32068 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
WB		1/2000. Detects a band of approximately 46 kDa (predicted molecular weight: 46 kDa).
IHC-P		1/25. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Application notes Is unsuitable for ICC/IF or IP.

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Function	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.
Tissue specificity	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.
Sequence similarities	Belongs to the peptidase C14A family.

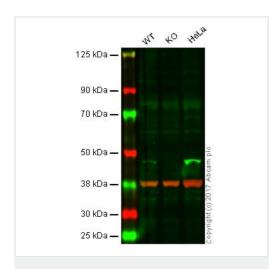
Developmental stage Expressed at low levels in fetal heart, at moderate levels in neonate heart, and at high levels in

adult heart.

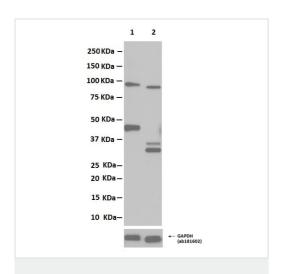
Contains 1 CARD domain.

Post-translationalCleavages at Asp-315 by granzyme B and at Asp-330 by caspase-3 generate the two activemodificationssubunits. Caspase-8 and -10 can also be involved in these processing events.

Images



Western blot - Anti-Caspase-9 antibody [E84] (ab32068)



Western blot - Anti-Caspase-9 antibody [E84] (ab32068)

Lanes:

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

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

Lane 3: HeLa whole cell lysate (40 µg).

Lanes 1 - 3 Merged signal (red and green). Green - ab32068 observed at 45 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32068 was shown to specifically recognize CASP9 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when CASP9 knockout samples were examined. Wild-type and CASP9 knockout samples were subjected to SDS-PAGE. Ab32068 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.

All lanes : Anti-Caspase-9 antibody [E84] (ab32068) at 1/1000 dilution

Lane 1 : Untreated HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HeLa treated with staurosporine 1uM for 4 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

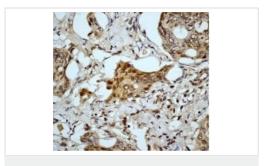
Predicted band size: 46 kDa

Additional bands at: 35 kDa, 37 kDa. We are unsure as to the

identity of these extra bands.

Exposure time: 3 minutes

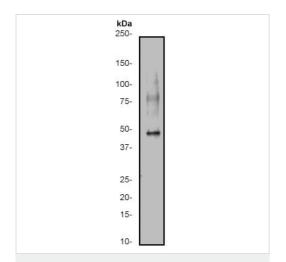
Blocking/dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Caspase-9 antibody
[E84] (ab32068)

Immunohistochemical analysis of paraffin-embedded human colon adenocarcinoma using anti-Pro-Caspase-9 RabMAb (ab32068).

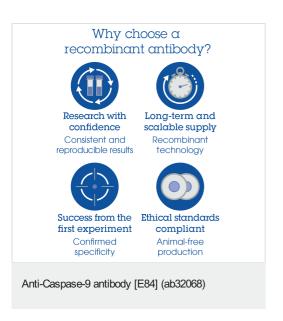
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-Caspase-9 antibody [E84] (ab32068)

Anti-Caspase-9 antibody [E84] (ab32068) at 1/2000 dilution + Jurkat cell lysate at 10 μg

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



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