abcam

ab219590

Anti-Caspase-9 antibody [E23] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	E23
Purification technique	Affinity purification Protein A
Specificity	This antibody should recognise both the pro-[40kDa] form and p35 cleaved form of Caspase-9.
Concentration	1.06 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human

Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	We recommend overnight incubation at 4°C.

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information CASP9

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 effector caspases caspase-3 (CASP3) or caspase-7 (CASP7). Promotes DNA damage-induced apoptosis in a ABL1/c-Abl-dependent manner. Proteolytically cleaves poly(ADP-ribose) polymerase (PARP). Cleaves BIRC6 following inhibition of BIRC6-caspase binding by DIABLO/SMAC (PubMed:36758105, PubMed:36758106). Isoform 2. Lacks activity is an dominant-negative inhibitor of caspase-9.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab219590 is the carrier-free version of ab32539.

Species reactivity

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

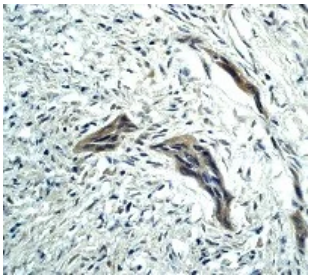
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

8 product images

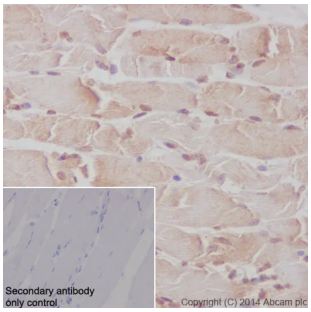


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

Caspase-9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Caspase-9 antibody

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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32539).

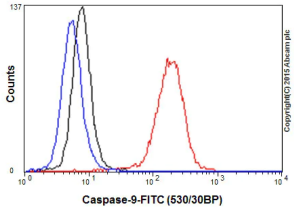
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

Caspase-9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Caspase-9 antibody

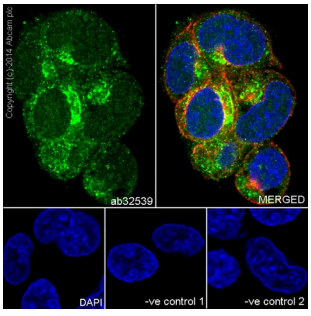
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle tissue labelling Caspase-9 with purified ab32539 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as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



Flow Cytometry (Intracellular) - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

Caspase-9 Flow Cytometry (Intracellular) staining using rabbit Anti-Caspase-9 antibody

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32539).



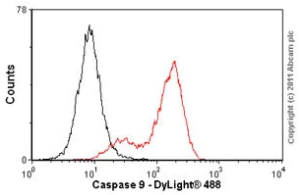
Immunocytochemistry/ Immunofluorescence - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

Caspase-9 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Caspase-9 antibody

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).

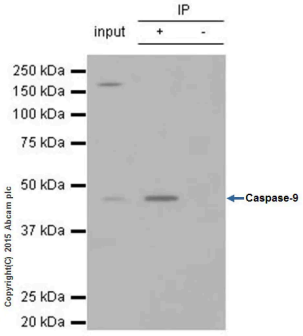
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32539).



Flow Cytometry (Intracellular) - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

Caspase-9 Flow Cytometry (Intracellular) staining using rabbit Anti-Caspase-9 antibody

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/1x10^6 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32539).



Immunoprecipitation - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

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.

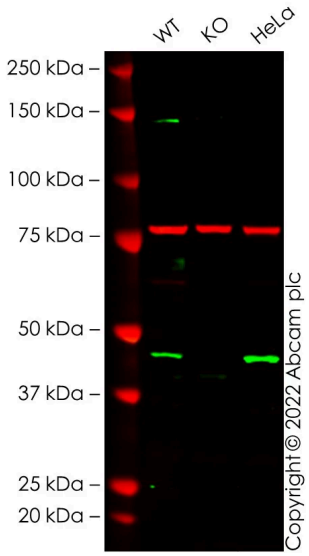
Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32539).

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

Predicted band size: 46 kDa

Observed band size: 46 kDa



Western blot - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

Caspase-9 Western blot staining using rabbit Anti-Caspase-9 antibody

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

Lane 1:
Wild-type THP-1 cell lysate at 20 µg

Lane 2:
CASP9 knockout THP-1 cell lysate at 20 µg

Lane 3:
HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 46 kDa

Observed band size: 45 kDa

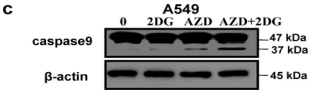


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Western blot - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

Caspase-9 western blot using anti-Caspase-9 antibody [E23] - BSA and Azide free ab219590. Publication image and figure legend from Jiang, S., Zou, Z., et al., 2015, PLoS One, PubMed 26176608.

ab219590 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab219590 please see the product overview.

mTOR inhibitors combined with glycolysis inhibitor induced apoptosis in lung cancer cells.(a) A549 cells were pretreated with 5 mM 2DG for 1 h, and then cells were treated with 8 µM rapamycin or 5 µM AZD2014 for additional 48 h respectively, cell apoptosis was then examined by using an Annexin V-FITC/PI staining assay kit. (b) A549 cells were pretreated with 5 mM 2DG for 1 h, and then treated with 5 µM AZD2014 for additional 48 h, as well as the cells were untreated and treated with 5 mM 2-DG or 5 µM AZD2014 alone for 48 h before harvesting cellular proteins. (c) A549 cells were pretreated with 5 mM 2DG for 1 h, and then treated with 5 µM AZD2014 for additional 48 h. Meanwhile, the cells were untreated and treated with 5 mM 2DG or 5 µM AZD2014 alone for 48 h before harvesting cellular proteins. All cell lysates were probed for the apoptosis–associated proteins such as BAX, BCL-2, cleaved PARP, cleaved caspase-3 and caspase 9 by Western blot. The

similar results were obtained from other two independent experiments, and data shown are the representatives of the three experiments.

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