abcam

ab32539

Anti-Caspase-9 antibody [E23]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
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	1.301 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/50 - 1/250

Notes	-

IP

Tested

Species	Human
Dilution info	1/80 - 1/100
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/10000
Notes	We recommend overnight incubation at 4°C.

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	**For unpurified use at 1/50.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	ab172730 - 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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

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

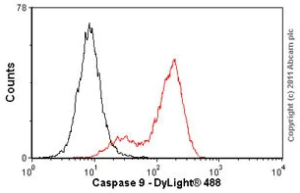
We do not recommend this combination. It is not covered by our product promise.

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.

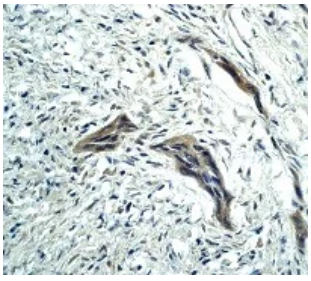
10 product images



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

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.

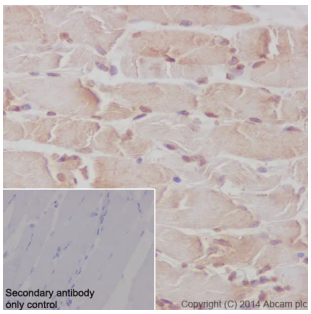


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-9 antibody [E23] (ab32539)

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.

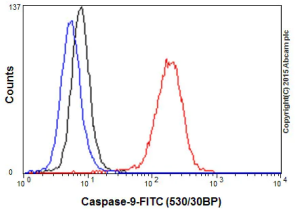
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

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

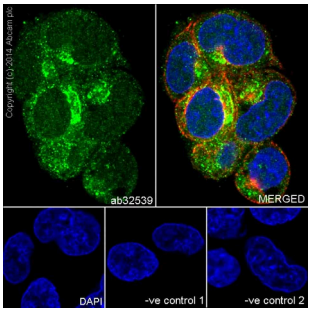
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle tissue labelling Caspase-9 with purified ab32539 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as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



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

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.

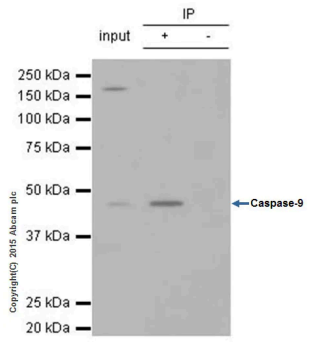


Immunocytochemistry/ Immunofluorescence - Anti-Caspase-9 antibody [E23] (ab32539)

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).



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

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.

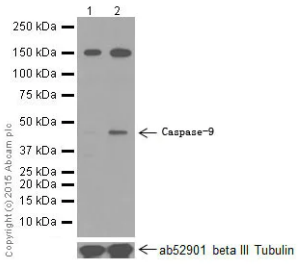
For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.
All lanes:
Immunoprecipitation - Anti-Caspase-9 antibody [E23] (ab32539)

Predicted band size: 46 kDa

Observed band size: 46 kDa



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

Blocking and dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Caspase-9 antibody [E23] (ab32539) at 1/1000 dilution

Lane 1:
HeLa whole cell lysate - treated with Camptothecin at 10 µg

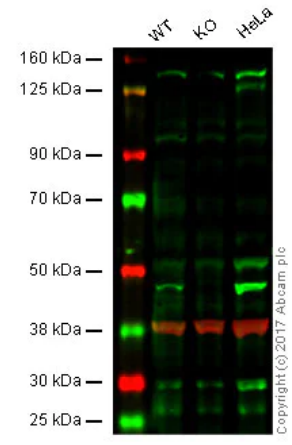
Lane 2:
HeLa whole cell lysate - untreated at 10 µg

Secondary

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

Predicted band size: 46 kDa

Observed band size: 35 kDa, 46 kDa



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

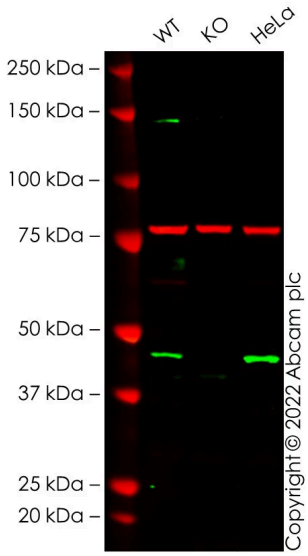
Caspase-9 Western blot staining using rabbit Anti-Caspase-9 antibody

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.

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

Predicted band size: 46 kDa



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

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

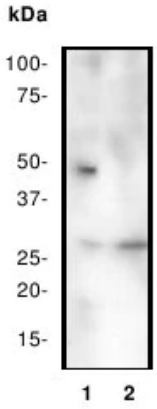
Lane 2:
CASP9 knockout THP-1 cell lysate at 20 µg

Lane 3:
HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 46 kDa

Observed band size: 45 kDa



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

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

Lane 1:
Jurkat cell lysate - untreated

Lane 2:
Jurkat cell lysate - treated with Camptothecin

Predicted band size: 46 kDa

Observed band size: 28 kDa, 48 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.