abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

43 References 8 Images

Overview

Product name	Anti-Caspase-9 antibody [E23] - BSA and Azide free
Description	Rabbit monoclonal [E23] to Caspase-9 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody should recognise both the pro-[40kDa] form and p35 cleaved form of Caspase-9.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: THP-1 and HeLa cell lysates. IHC-P: Human skeletal muscle and Human cervical carcinoma tissues. ICC/IF: HepG2 cells. Flow Cyt (intra): K562 cells. IP: HeLa whole cell lysate.
General notes	ab219590 is the carrier-free version of **ab32539**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E23
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab219590 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
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 46 kDa. We recommend overnight incubation at 4°C.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

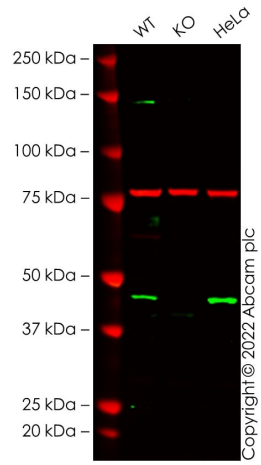
Target

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. Contains 1 CARD domain.
Developmental stage	Expressed at low levels in fetal heart, at moderate levels in neonate heart, and at high levels in adult heart.
Post-translational modifications	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.

Images



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

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

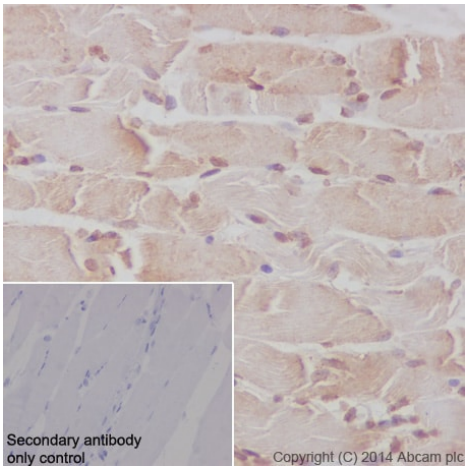
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : CASP9 knockout THP-1 cell lysate
Lane 3 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

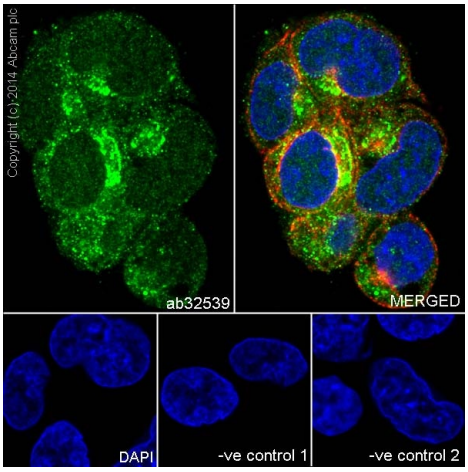
False colour image of Western blot: Anti-Caspase-9 antibody [E23] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32539** was shown to bind specifically to Caspase-9. A band was observed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



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

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**).



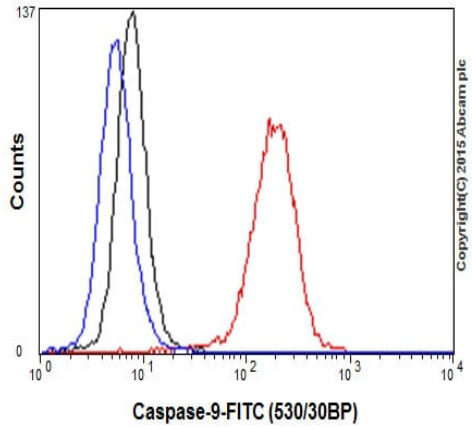
Immunocytochemistry/ Immunofluorescence - Anti-Caspase-9 antibody [E23] - BSA and Azide free (ab219590)

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).

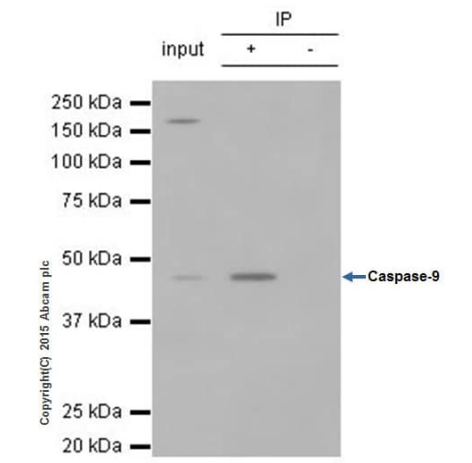
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32539**).



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

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**).



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.

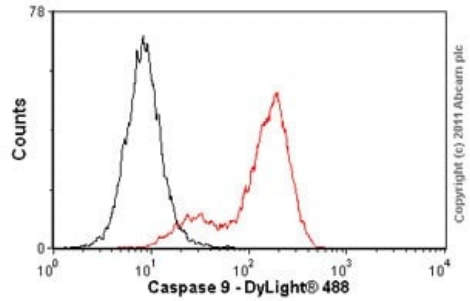
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32539** in HeLa whole cell lysate.

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.

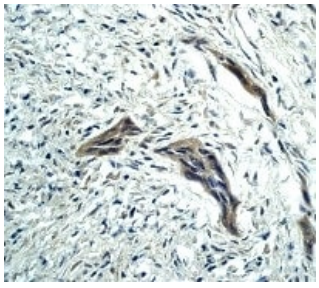
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32539**).



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

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**).



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

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.

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

(ab219590)

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