abcam

Product datasheet

Anti-Caspase-9 antibody [EPR18107] - BSA and Azide free ab222231

KO VALIDATED Recombinant RabMAb

1 References 5 Images

Overview

Product name	Anti-Caspase-9 antibody [EPR18107] - BSA and Azide free
Description	Rabbit monoclonal [EPR18107] to Caspase-9 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IP, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa and THP-1 whole cell lysates. IHC-P: Human cervix carcinoma tissue. ICC/IF: HeLa cells. IP: HeLa treated with staurosporine 1uM for 4 hours whole cell lysate.
General notes	ab222231 is the carrier-free version of **ab202068**. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18107
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222231 in the following tested applications.

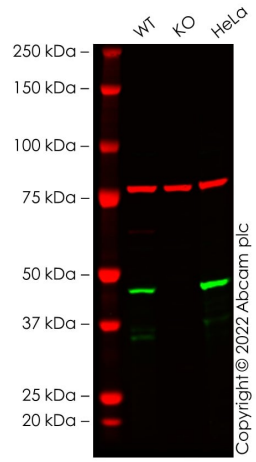
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 46, 35, 37 kDa (predicted molecular weight: 46 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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 [EPR18107] - BSA and Azide free (ab222231)

All lanes : Anti-Caspase-9 antibody [EPR18107] (**ab202068**) at 1/2000 dilution

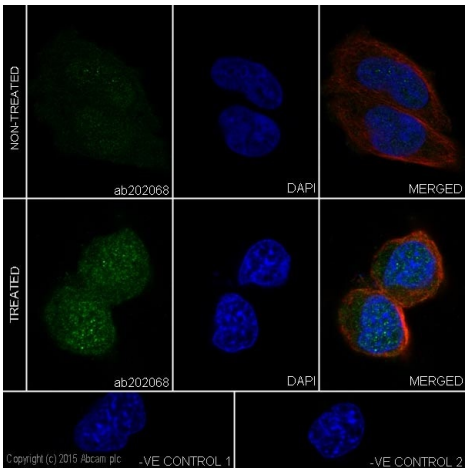
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : CASP9 knockout THP-1 cell lysate
Lane 3 : HeLa cell lysate

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 [EPR18107] staining at 1/2000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab202068** 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.



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-9 antibody [EPR18107] - BSA and Azide free (ab222231)

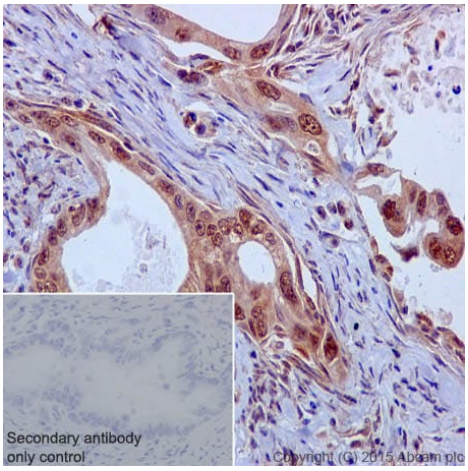
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Caspase-9 with **ab202068** at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green).

Confocal image showing cytoplasmic and nuclear staining on HeLa cell line. The expression increased after treatment with staurosporine (1uM) for 4 hours.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
-ve control 1: **ab202067** at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab202068**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-9 antibody [EPR18107] - BSA and Azide free (ab222231)

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling Caspase-9 with **ab202068** at 1/300 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution.

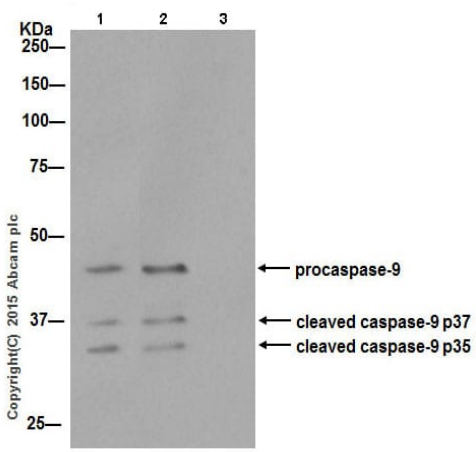
Cytoplasmic and nuclear staining on Human cervix carcinoma tissue is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab202068**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-Caspase-9 antibody [EPR18107] - BSA and Azide free (ab222231)

Caspase-9 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) treated with staurosporine 1uM for 4 hours whole cell lysate with **ab202068** at 1/80 dilution.

Western blot was performed from the immunoprecipitate using **ab202068** at 1/1000 dilution.

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG was used as secondary antibody at 1/1500 dilution.

Lane 1: HeLa treated with staurosporine 1uM for 4 hours whole cell lysate10 µg (Input).

Lane 2: **ab202068** IP in HeLa treated with staurosporine 1uM for 4 hours whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab202068** in HeLa treated with staurosporine 1uM for 4 hours whole cell lysate. Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab202068**).

Anti-Caspase-9 antibody [EPR18107] - BSA and Azide free (ab222231)

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