abcam

Product datasheet

Anti-pro Caspase-3 antibody [E83-103] - BSA and Azide free ab238440

KO VALIDATED Recombinant RabMAb

1 References 5 Images

Overview

Product name	Anti-pro Caspase-3 antibody [E83-103] - BSA and Azide free
Description	Rabbit monoclonal [E83-103] to pro Caspase-3 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody only detects pro-form (35kD) of caspase 3, and does not recognize any cleaved caspases.
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide within Human pro Caspase-3 (N terminal). The exact sequence is proprietary. A synthetic peptide corresponding to residues following Ser29 of human caspase-3 (N-terminus of p17 subunit).
Positive control	WB: Wild-type HAP1 whole cell lysate. IHC-P: Human colon adenocarcinoma tissue. ICC/IF: HeLa cells. Flow Cyt (intra): Jurkat cells.
General notes	ab238440 is the carrier-free version of **ab32499**. 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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this 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: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E83-103
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab238440 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.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 35 kDa (predicted molecular weight: 31 kDa).
ICC/IF		Use at an assay dependent concentration.

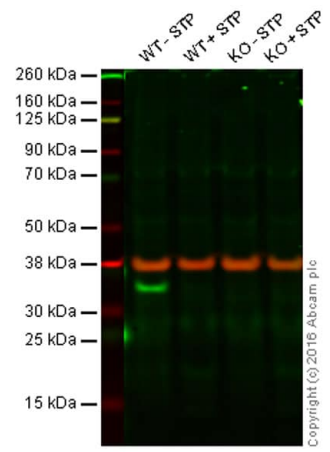
Target

Relevance Caspases are a family of cysteine proteases that are key mediators of programmed cell death or apoptosis. The precursor form of all caspases is composed of a prodomain, and large and small catalytic subunits. The active forms of caspases are generated by several stimuli including ligand-receptor interactions, growth factor deprivation and inhibitors of cellular functions. All known caspases require cleavage adjacent to aspartates to liberate one large and one small subunit, which associate into a2b2 tetramer to form the active enzyme. Gene for Caspase 3 also known

as Yama, CPP32, and apopain codes for a 32-kDa protein. Caspase 3 cleaves the death substrate poly(ADP-ribose) polymerase (PARP) to a specific 85 kDa form observed during apoptosis and is inhibitable by the CrmA protein. Other Caspase 3 substrates include DNA-PK, actin, GAS2, and procaspase-6, etc. Caspase 3 is activated by cleavage events at Asp-28/Ser-29 (between N-terminal pro-domain) and Asp-175/Ser-176 (between large and small subunits) to generate a large subunit of 17-kDa and a small subunit of 12-kDa.

Cellular localization Cytoplasmic

Images

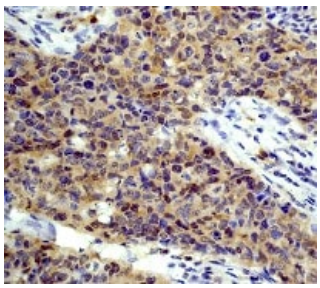


Western blot - Anti-pro Caspase 3 antibody [E83-103] - BSA and Azide free (ab238440)

Lane 1: Wild-type HAP1 cell lysate
Lane 2: Wild-type HAP1 cell lysate + Staurosporine (1µM for 4h)
Lane 3: Caspase-3 knockout HAP1 cell lysate
Lane 4: Caspase-3 knockout HAP1 cell lysate + Staurosporine (1µM for 4h)
Lanes 1 - 4: Merged signal (red and green). Green - **ab32499** observed at 35 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32499 was shown to specifically react with pro Caspase 3 when Caspase 3 knockout samples were used. Wild-type and Caspase 3 knockout samples (± Staurosporine treatment) were subjected to SDS-PAGE. **ab32499** and **ab8245** (loading control to GAPDH) were diluted to 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab32499**).

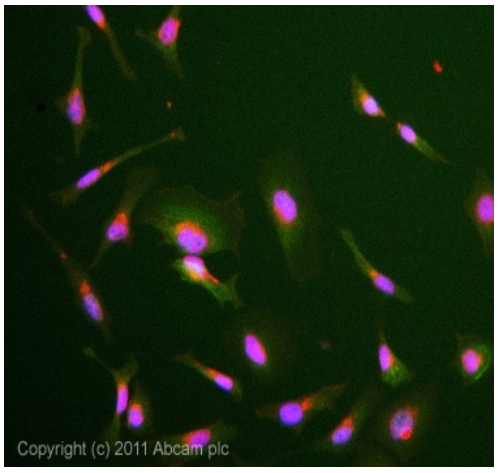


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-pro Caspase 3 antibody [E83-103] - BSA and Azide free (ab238440)

Immunohistochemical analysis of paraffin-embedded human colon adenocarcinoma **ab32499** at 1/250 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab32499**)

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

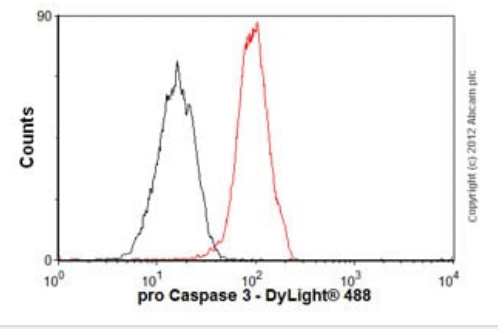


Immunocytochemistry/ Immunofluorescence - Anti-pro Caspase 3 antibody [E83-103] - BSA and Azide free (ab238440)

ICC/IF image of **ab32499** stained HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed in 4% formaldehyde (10 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (**ab32499**, 5 µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, anti-rabbit DyLight® 488 used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 µM.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab32499**)



Flow Cytometry (Intracellular) - Anti-pro Caspase-3 antibody [E83-103] - BSA and Azide free (ab238440)

Overlay histogram showing Jurkat (Human T cell leukemia cell line from peripheral blood) cells stained with **ab32499** (red line).

The cells were fixed with 80% 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 (**ab32499**, 1/50 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 IgG (monoclonal) (1µg/1 x 10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab32499**)

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