abcam

Product datasheet

Anti-Cleaved Caspase-3 antibody [E83-77] - BSA and Azide free ab208003

KO VALIDATED Recombinant RabMAb

4 References 3 Images

Overview

Product name	Anti-Cleaved Caspase-3 antibody [E83-77] - BSA and Azide free
Description	Rabbit monoclonal [E83-77] to Cleaved Caspase-3 - BSA and Azide free
Host species	Rabbit
Specificity	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. This antibody only detects the active (cleaved) form of Caspase-3 and does not recognize the pro form of Caspase-3. PubMedID 19789217 describes the detection of human cells injected into mice. The pro-caspase-3 is cleaved only when apoptosis event occurs. So, in order to detect active Caspase-3, we strongly suggest to induce your samples into apoptotic pathway.
Tested applications	**Suitable for:** WB, ICC/IF **Unsuitable for:** Flow Cyt,IHC-P or IP
Species reactivity	**Reacts with:** Human, Recombinant fragment
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Jurkat cell lysate, treated wild type HeLa cell lysate. ICC/IF: Hela cells.
General notes	ab208003 is the carrier-free version of **ab32042**. 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. 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	E83-77
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab208003 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
WB		Use at an assay dependent concentration. Detects a band of approximately 17 kDa (predicted molecular weight: 32 kDa).
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt,IHC-P or IP.

Target

Function Involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis it proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp--Gly-217' bond. Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Cleaves and activates caspase-6, -7 and -9. Involved in the cleavage of huntingtin. Triggers cell adhesion in sympathetic neurons through RET cleavage.

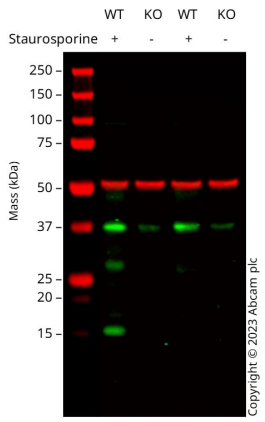
Tissue specificity Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system.

Sequence similarities Belongs to the peptidase C14A family.

Post-translational modifications Cleavage by granzyme B, caspase-6, caspase-8 and caspase-10 generates the two active subunits. Additional processing of the propeptides is likely due to the autocatalytic activity of the activated protease. Active heterodimers between the small subunit of caspase-7 protease and the large subunit of caspase-3 also occur and vice versa.
S-nitrosylated on its catalytic site cysteine in unstimulated human cell lines and denitrosylated upon activation of the Fas apoptotic pathway, associated with an increase in intracellular caspase activity. Fas therefore activates caspase-3 not only by inducing the cleavage of the caspase

	zymogen to its active subunits, but also by stimulating the denitrosylation of its active site thiol.
Cellular localization	Cytoplasm.

Images



Western blot - Anti-Cleaved Caspase-3 antibody [E83-77] - BSA and Azide free (ab208003)

All lanes : Anti-Cleaved Caspase-3 antibody [E83-77] (**ab32042**) at 1/500 dilution

Lane 1 : Wild-type HeLa Treated Staurosporine (2uM, 4h) cell lysate
Lane 2 : CASP3 knockout HeLa Treated Staurosporine (2uM, 4h) cell lysate
Lane 3 : Wild-type HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate
Lane 4 : CASP3 knockout HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate

Lysates/proteins at 20 µg per lane.

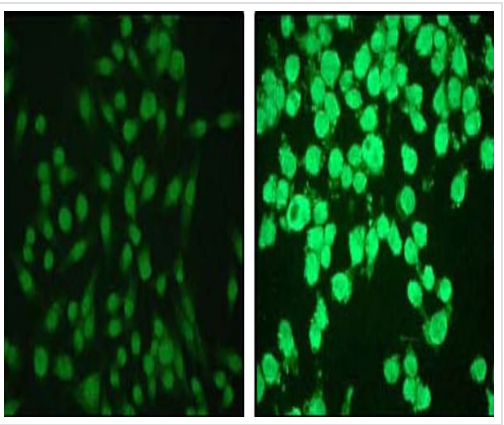
Performed under reducing conditions.

Predicted band size: 32 kDa
Observed band size: 16,28 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32042**).

Anti-CASP3 antibody [E83-77] (**ab32042**) staining at 1/500 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32042** was shown to bind specifically to CASP3. A band was observed at 16/28 kDa in treated wild-type HeLa cell lysates with no signal observed at this size in CASP3 knockout cell line. To generate this image, wild-type and CASP3 knockout HeLa 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Cleaved Caspase-3 antibody [E83-77] - BSA and Azide free (ab208003)

Ab32042, at dilution of 1/100, staining HeLa cells by Immunofluorescence.
Left image: control.
Right image: staurosporine treated.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32042**).

Anti-Cleaved Caspase-3 antibody [E83-77] - BSA and Azide free (ab208003)

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