abcam

Product datasheet

Anti-Cleaved Caspase-3 antibody [E83-77] ab32042

KO VALIDATED Recombinant RabMAb

27 Abreviews 463 References 7 Images

Overview

Product name	Anti-Cleaved Caspase-3 antibody [E83-77]
Description	Rabbit monoclonal [E83-77] to Cleaved Caspase-3
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 within Human Cleaved Caspase-3 aa 1-100 (N terminal). The exact sequence is proprietary. A synthetic peptide corresponding to residues following Ser29 of human Caspase 3 (N terminus of p17 subunit). Database link: **P42574**
Positive control	WB: Wild type HAP1 + 2uM Staurosporine (**ab146588**) for 24 hours, whole cell lysate; Jurkat cell lysate (camptothecin treated); HeLa cell lysate (staurosporine treated). ICC/IF: Hela cells (staurosporine treated); Human Vascular endothelial cells.
General notes	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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E83-77
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32042 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	★★★★☆ (7)	1/500. Detects a band of approximately 17 kDa (predicted molecular weight: 32 kDa). 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.
ICC/IF	★★★★☆ (5)	1/100 - 1/250.

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.

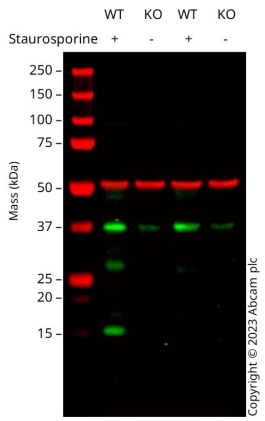
Tissue specificity Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system.

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] (ab32042)

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

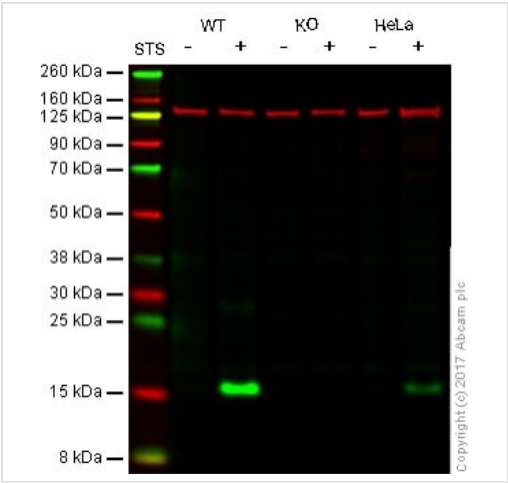
Lane 1 : Wild-type HeLa Treated Staurosporine (2uM, 4h) cell lysate
Lane 2 : CASP3 knockout HeLa Treated Staurosporine (2uM, 4h) cell lysate
Lane 3 : Wild-type HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate
Lane 4 : CASP3 knockout HeLa Vehicle Control Staurosporine (0uM, 4h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Western blot: 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.

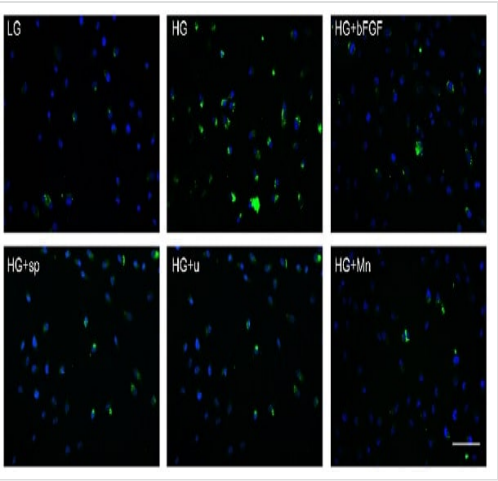


Western blot - Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042)

Lane 1: Wild type HAP1 + DMSO for 24 hours, whole cell lysate (20 µg)
Lane 2: Wild type HAP1 + 2uM Staurosporine (**ab146588**) for 24 hours, whole cell lysate (20 µg)
Lane 3: HAP1 CASP3 KO + DMSO for 24 hours, whole cell lysate (20 µg)
Lane 4: HAP1 CASP3 KO + 2uM Staurosporine (**ab146588**) for 24 hours, whole cell lysate (20 µg)
Lane 5: HeLa + DMSO for 24 hours, whole cell lysate (20 µg)
Lane 6: HeLa + 2uM Staurosporine (**ab146588**) for 24 hours, whole cell lysate (20 µg)
Lanes 1 - 6: Merged signal (red and green). Green - ab32042 observed at 17 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

ab32042 was shown to specifically react with CASP3 (Caspase-3) when CASP3 (Caspase-3) knockout samples were used. HAP1 wild-type and CASP3 (Caspase-3) knockout samples were subjected to SDS-PAGE. Ab32042 and **ab130007** (Mouse anti vinculin loading control) were incubated overnight at 4°C at 500 dilution and 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.

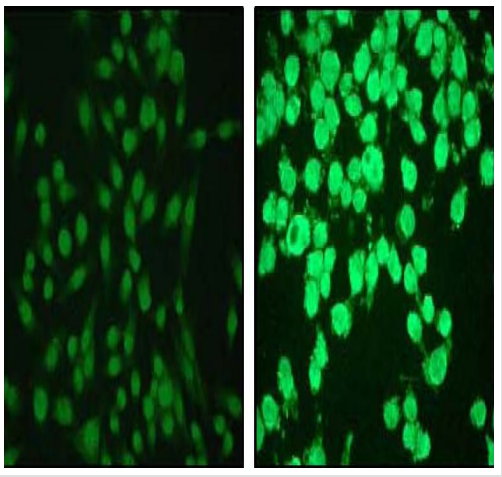
Cells were grown to confluency prior to treatment.



Immunocytochemistry/ Immunofluorescence - Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042)

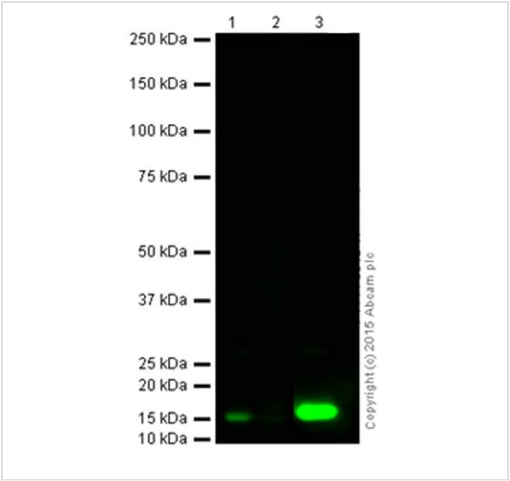
High-glucose induces apoptosis in human Vascular endothelial cells (VECs).

Apoptotic responses in VEC were analyzed by detection of cleaved-Caspase-3 immunofluorescence using ab32042. Cells were treated with low glucose (LG) or high glucose (HG) for 72 hours before treated with 100 ng/mL bFGF, 1 μM sp600125 (sp), or 1 μM U0126 (U) or 10 μM MnTmPyP for 1 hour. Bar = 100 μm.



Immunocytochemistry/ Immunofluorescence - Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042)

Ab32042, at dilution of 1/100, staining HeLa (human epithelial cell line from cervix adenocarcinoma) cells by Immunofluorescence.
Left image: control.
Right image: staurosporine treated.



Western blot - Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042)

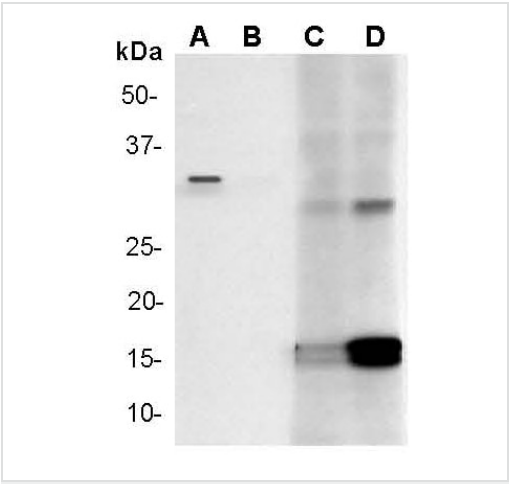
All lanes : Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042) at 1/500 dilution

Lane 1 : HeLa Whole Cell Lysate (2 uM Staurosporine, 4Hr) at 20 µg
Lane 2 : HeLa Whole Cell Lysate (untreated) at 20 µg
Lane 3 : Cleaved Caspase 3 (recombinant protein) at 0.1 µg

Secondary
All lanes : 800CW Goat Anti-Rabbit IgG at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 32 kDa
Additional bands at: 17 kDa (possible mature (processed) protein)



Western blot - Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042)

Lane 1 : anti Pro Caspase 3 at 1/10000 dilution
Lane 2 : anti Pro Caspase 3 at 1/10000 dilution
Lanes 3-4 : Anti-Cleaved Caspase-3 antibody [E83-77] (ab32042) at 1/500 dilution

Lane 1 : Jurkat (human T cell leukemia cell line from peripheral blood) cell lysate
Lanes 2 & 4 : Jurkat cell lysate + Camptothecin
Lane 3 : Jurkat cell lysate

Predicted band size: 32 kDa
Observed band size: 17 kDa
Additional bands at: 30 kDa. We are unsure as to the identity of these extra bands.

Anti-Cleaved Caspase-3 antibody [E83-77]

(ab32042)

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