Overview

Product name: Anti-active Caspase-3 antibody [E83-77] ab32042

Description: Rabbit monoclonal [E83-77] to active 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: IHC-Fr, WB, ICC/IF
- Unsuitable for: Flow Cyt or IP

Species reactivity: Reacts with: Human

Immunogen: Synthetic peptide within Human active 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:

Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents This product is a recombinant rabbit monoclonal antibody.

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
Clonality: Monoclonal
Clone number: E83-77
Isotype: IgG

Preservative: 0.01% Sodium azide
Constituents: 49% PBS, 50% Glycerol, 0.05% BSA

Applications

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>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.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100 - 1/250.</td>
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Application notes

Is unsuitable for Flow Cyt 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

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

Apoptotic responses in VEC were analyzed by detection of active-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.

Lane 1: Wild type HAP1 + DMSO for 24 hours, whole cell lysate (20 μg)
Lane 2: Wild type HAP1 + 2μM 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 + 2μM 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 + 2μM 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, ab8245, 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 ab8245 (Mouse anti GAPDH 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-active 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-active Caspase-3 antibody [E83-77] (ab32042)

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

Lane 1: HeLa Whole Cell Lysate (2 µM Staurosporine, 4Hr) at 20 µg
Lane 2: HeLa Whole Cell Lysate (untreated) at 20 µg
Lane 3: Active 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-active 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-active 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

why is the actual band size different from the predicted?
Additional bands at: 30 kDa. We are unsure as to the identity of these extra bands.
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