Anti-Caspase-3 antibody [E87] ab32351

Overview

Product name: Anti-Caspase-3 antibody [E87]

Description: Rabbit monoclonal [E87] to Caspase-3

Host species: Rabbit

Specificity: This antibody is specific for the pro form and the p17 cleaved form of human Caspase-3.

Tested applications: Suitable for: ICC/IF, WB, IHC-P, Flow Cyt, IP

Species reactivity: Reacts with: Human

Immunogen: Synthetic peptide within Human Caspase-3 aa 50-150. The exact sequence is proprietary.

Database link: P42574


General notes: A trial size is available to purchase for this antibody.

Rat reactivity: We have preliminary internal testing data indicating this antibody does not react with mouse caspase 3. Internal testing and customer reports also indicate non-reactivity with rat caspase 3, contrary to earlier positive reports. Please contact us for more information.

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

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form: Liquid


Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity | Protein A purified
Clonality | Monoclonal
Clone number | E87
Isotype | IgG

### Applications

Our **Abpromise guarantee** covers the use of ab32351 in the following tested applications.

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

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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>ICC/IF</td>
<td>🌟🌟🌟🌟🌟</td>
<td>1/500. <strong>For unpurified, use 1/25.</strong></td>
</tr>
<tr>
<td>WB</td>
<td>🌟🌟🌟🌟🌟</td>
<td>1/5000. Detects a band of approximately 35 kDa (predicted molecular weight: 32 kDa).</td>
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<tr>
<td>IHC-P</td>
<td>🌟🌟🌟🌟🌟</td>
<td>1/25 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See <strong>IHC antigen retrieval protocols</strong>.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>🌟🌟🌟🌟🌟</td>
<td>1/180 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IP</td>
<td>🌟🌟🌟🌟🌟</td>
<td>1/10 - 1/50.</td>
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### 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. |
| 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. |
**Western blot - Anti-Caspase-3 antibody [E87] (ab32351)**

**All lanes** : Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution

**Lane 1** : DMSO control wild-type HAP1 whole cell lysate

**Lane 2** : Staurosporine treated wild-type HAP1 whole cell lysate

**Lane 3** : DMSO control CASP3 knockout HAP1 whole cell lysate

**Lane 4** : Staurosporine treated CASP3 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size**: 32 kDa

**Lanes 1 - 4**: Merged signal (red and green). Green - ab32351 observed at 31 kDa. Red - loading control, ab130007, observed at 130 kDa.

ab32351 was shown to recognize Caspase 3 in wild-type HAP1 cells as signal was lost at the expected MW in HAP1 Staurosporine Treated (CASP3) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and HAP1 Staurosporine Treated (CASP3) knockout samples were subjected to SDS-PAGE. ab32351 and ab130007 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.
Immunofluorescence staining of Jurkat cells with purified ab32351 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab32351 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.

Unpurified ab32351, at a 1/25 dilution, staining Caspase-3 in paraffin embedded human cervical carcinoma tissue by Immunohistochemistry.

**All lanes**: Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution (purified)

**Lane 1**: untreated Jurkat cell lysate

**Lane 2**: Jurkat treated with staurosporine

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

**Predicted band size**: 32 kDa

**Observed band size**: 17.35 kDa

_why is the actual band size different from the predicted?_
Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST

**Western blot - Anti-Caspase-3 antibody [E87] (ab32351)**

**All lanes**: Anti-Caspase-3 antibody [E87] (ab32351) at 1/5000 dilution (purified)

**Lane 1**: Ramos cell lysate

**Lane 2**: HEK293 cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

**Predicted band size**: 32 kDa

**Observed band size**: 35 kDa

**why is the actual band size different from the predicted?**

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caspase-3 antibody [E87] (ab32351)**

Immunohistochemical staining of paraffin embedded human tonsil with purified ab32351 at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.
ab32351 (purified) at 1/50 immunoprecipitating Culin 1 in 10 μg HeLa whole cell lysate (Lanes 1 and 2, observed at 35 kDa). Lane 3 - PBS. For western blotting, VeriBlot for IP (HRP) (ab131366) was used as the secondary antibody (1/1500). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST

Overlay histogram showing Ramos cells fixed in 4% PFA and stained with purified ab32351 at a dilution of 1 in 180 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).

Carried out with unpurified antibody. Lane 1 = Caspase 3 protein (Active) (ab52314) ? 20 ng. Lane 2 = Caspase 9 protein (Active) (ab52203) ? 20 ng. Lane 3 = Extract of HeLa cells treated with vehicle (ab136806) ? 20 ug. Lane 4 = Extract of HeLa cells treated with staurosporine (ab136806) ? 20 ug. SDS PAGE performed under reducing conditions (100 mM DTT ? Sample heated at 50? C). Primary: Lanes 1-4: Anti Caspase 3 antibody (ab32351) at 1:1000 dilution. Secondary: Lanes 1-4: Goat anti rabbit IgG(H&L)-HRP at 1:10000. Development: ECL for 10 min exposure. Blocking: in 5% Milk + PBS overnight at 4 C. Primary antibody: in 5% Milk + PBS for 2 hours at RT. Secondary antibody: in 5% Milk + PBS for 2 hours at RT. Predicted band size: 32 kDa and 17 kDa. Observed band size: 32 kDa and 17 kDa.
Overlay histogram showing HeLa cells stained with unpurified ab32351 (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 (ab32351, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (ab172730, 0.1μg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

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