## Anti-Cleaved Caspase-3 antibody ab2302

**Overview**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-Cleaved Caspase-3 antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit polyclonal to Cleaved Caspase-3</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Specificity</td>
<td>The antibody preferentially recognizes the p17 fragment of the active Caspase-3. Under the conditions we tested, the antibody did not detect the precursor form. Under other conditions (such as those used in PMC2206181), this antibody has been found to detect both the pro and active forms of Caspase-3. Although some customers have used this antibody successfully in mouse and rat, we do not batch test in these species and so cannot guarantee that it will work in them.</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: IHC-FoFr, IHC-P, IHC-Fr, WB</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Rabbit, Human, Quail Predicted to work with: Mouse, Rat</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Synthetic peptide corresponding to Human Cleaved Caspase-3. Synthetic peptide mapping to the N-terminus adjacent to the cleavage site of human caspase-3 Database link: P42574 (Peptide available as ab38283)</td>
</tr>
<tr>
<td>Positive control</td>
<td>Camptothecin (2 µM) treated Jurkat cells. If no signal is observed a time course may be required to identify maximal caspase activity.</td>
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<tr>
<td>General notes</td>
<td>Caspases are synthesized as inactive pro-enzymes that are processed to active form in cells undergoing apoptosis. Caspase 3 has been extensively studied and implicated to play an important role in apoptosis. Active caspase 3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cells (e.g., PARP). This affinity purified antibody recognizing the active forms of caspase-3 provides a new tool for identifying apoptotic cell populations in both tissue sections and cultured cells. Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077). See other anti-rabbit secondary antibodies that can be used with this antibody.</td>
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</table>

**Properties**

<table>
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<tr>
<th>Form</th>
<th>Liquid</th>
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</table>
| Storage instructions | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -
80°C. Avoid freeze / thaw cycle.

Storage buffer
Preservative: 0.01% Thimerosal (merthiolate)
Constituents: PBS, 30% Glycerol, 0.5% BSA

Purity
Immunogen affinity purified

Primary antibody notes
Caspases are synthesized as inactive pro-enzymes that are processed to active form in cells undergoing apoptosis. Caspase 3 has been extensively studied and implicated to play an important role in apoptosis. Active caspase 3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cells (e.g., PARP). This affinity purified antibody recognizing the active forms of caspase-3 provides a new tool for identifying apoptotic cell populations in both tissue sections and cultured cells.

Clonality
Polyclonal

Isotype
IgG

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

Applications
Our Abpromise guarantee covers the use of ab2302 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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<tbody>
<tr>
<td>IHC-FoFr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 18797916</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 5 - 20 µg/ml. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 10 - 20 µg/ml.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa. Can be blocked with Human Caspase-3 peptide (ab38283).</td>
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</table>
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**

IHC image of cleaved caspase-3 staining in human tonsil formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab2302, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

**Western blot**

Anti-Cleaved Caspase-3 antibody (ab2302)

All lanes : Anti-Cleaved Caspase-3 antibody (ab2302) at 1 µg/ml

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.

**Additional bands at:** 17 kDa (possible mature (processed) protein), 24 kDa, 35 kDa. We are unsure as to the identity of these extra bands.
Immunohistochemical detection (on formaldehyde/PFA-fixed paraffin-embedded sections) of cleaved Caspase-3 antibody (ab2302) on Quail Tissue sections (Quail E6/7 developing DRGs Sagittal section). Antigen retrieval step: Heat mediated. Blocking step: 1% BSA for 10 mins RT. Primary Antibody ab2302 incubated at 1/50 for at RT. Secondary Antibody: Biotin conjugated goat anti rabbit IgG (1/300).

ab2302 staining cleaved Caspase-3 in human ulcerative colitis, colon tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed using the HOPE method and permeabilized. Samples were incubated with primary antibody (1/20) for 1 hour at 25°C. A Biotin-SP-conjugated donkey anti-rabbit IgG polyclonal (1/800) was used as the secondary antibody.

Anti-Cleaved Caspase-3 antibody (ab2302) at 1 µg/ml + Camptothecin (2 µM) treated Jurkat cells.
Lung sections were stained with caspase antibody ab2302. Caspase-3 positive cells are shown in brown (DAB). This picture was kindly supplied as part of a customer review.

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