Technical Data Sheet

Purified Rabbit Anti- Active Caspase-3

Product Information

Material Number: 559565
Alternate Name: CPP32; Yama; Apopain
Size: 25 µg
Concentration: 0.5 mg/ml
Clone: C92-605
Immunogen: Human Active Caspase-3 Fragment
Isotype: Rabbit IgG
Reactivity: QC Testing: Human
Tested in Development: Mouse
Target MW: 20 kD & 17 kD
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The caspase family of cysteine proteases plays a key role in apoptosis and inflammation. Caspase-3 is a key protease that is activated during the early stages of apoptosis and, like other members of the caspase family, is synthesized as an inactive pro-enzyme that is processed in cells undergoing apoptosis by self-proteolysis and/or cleavage by another protease. The processed forms of caspases consist of large (17-22 kDa) and small (10-12 kDa) subunits which associate to form an active enzyme. Active caspase-3, a marker for cells undergoing apoptosis, consists of a heterodimer of 17 and 12 kDa subunits which is derived from the 32 kDa pro-enzyme. Active caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cytoplasm, e.g., D4-GDI and Bcl-2, and in the nucleus (e.g. PARP). This antibody has been reported to specifically recognize the active form of caspase-3 in human and mouse cells. It has not been reported to recognize the pro-enzyme form of caspase-3.

Immunoprecipitation/Western blot analysis of caspase-3 from apoptotic and non-apoptotic cell lysates. Jurkat cells (human T-cell leukemia, ATCC TIB-152) were left untreated (left panel) or were treated with 6 µM camptothecin for 5 hr to induce apoptosis (right panel). Immunoprecipitation: Cell lysates were immunoprecipitated with 0.25 - 2 µg/ml of the rabbit anti- active caspase-3 antibody [clone C92-605, lanes 1 and 8 (2 µg), lanes 2 and 9, (1 µg), lanes 3 and 10 (0.5 µg), and lanes 4 and 11 (0.25 µg)], 1 - 2 µg/ml of a mAb recognizing both pro and active caspase-3 (Cat. No. 610322), lanes 5 and 12 (2 µg), lanes 6 and 13 (2 µg), lanes 6 and 13 (1 µg) or 1 µg/ml of a rabbit IgG isotype control [Jackson Immunoresearch (Cat. No. 011-00000-3), lanes 7 and 14]. Western blot: Caspase-3 was detected by western blot analysis with an antibody that recognizes both pro- (32 kD) and active (20 and 17 kD, reflecting the presence or absence of the caspase-3 pro-domain) caspase-3 [lanes 1-14]. The results also show that the rabbit anti-active caspase-3 antibody (clone C92-605) immunoprecipitated only the active form of caspase-3 (lanes 8-11) as compared to the mouse anti-human caspase-3 antibody (Cat. No. 610322) which immunoprecipitated both the pro and active forms of caspase-3 (lanes 12 and 13).

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Flow cytometric analysis for active caspase-3. Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were left untreated (left panel) or treated with 4 µM of camptothecin for 5 hr to induce apoptosis (right panel). Cells were fixed and permeabilized using the BD Cytofix/Cytoperm™ Kit (Cat. No. 554714) for 20 min at room temperature (RT), pelleted and washed with BD Perm/Wash™ buffer (component of Cat. No. 554714). Cells were subsequently stained with the rabbit anti-active caspase-3 antibody (clone C92-605) at 0.25 µg/1x10^6 cells for 20 min at RT in the dark. Afterwards, cells were washed in BD Perm/Wash™ buffer and stained with FITC donkey anti-rabbit IgG (Cat. No. 711-096-152; Jackson ImmunoResearch) using 0.25 µg/sample. Cells were then washed and resuspended in BD Perm/Wash™ buffer before analyzing by flow cytometry. The results show that untreated cells were negative for caspase-3 (M2, left panel); whereas more than 50% of the treated cells were positive for caspase-3 staining (M2, right panel).

Immunohistochemical staining for active caspase-3. Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were left untreated (top left quadrant) or treated for 4 hr with 6 µM camptothecin to induce apoptosis (top right quadrant). Mouse liver samples were either left untreated (bottom left quadrant) or treated with anti-mouse Fas mAb (100 µg in 250 µl of PBS injected i.p.; Cat. No. 554254) to induce apoptosis (bottom right quadrant), and sacrificed after 6 hr. Cytospins of Jurkat cells or frozen mouse liver tissue sections were acetone-fixed and stained with the rabbit anti-active caspase-3 antibody (clone C92-605), a biotin goat anti-rabbit secondary antibody and then with HRP-streptavidin. Staining was visualized with a DAB chromogen. Active caspase-3 staining was almost exclusively identified in cells or tissues induced to undergo apoptosis.

Application Notes

Application

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Recommended Assay Procedure:

**Bioimaging:** Please refer to [http://www.bdbiosciences.com/support/resources/bioimaging/index.jsp](http://www.bdbiosciences.com/support/resources/bioimaging/index.jsp)

**Suggested Companion Products**

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<tr>
<td>554254</td>
<td>Purified NA/LE Hamster Anti-Mouse CD95</td>
<td>0.5 mg</td>
<td>Jo2</td>
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<tr>
<td>554714</td>
<td>BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit</td>
<td>250 Tests</td>
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<tr>
<td>610322</td>
<td>Purified Mouse Anti-Human Caspase-3</td>
<td>50 µg</td>
<td>19/Caspase-3/CPP32</td>
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<tr>
<td>550578</td>
<td>Human Active Caspase-3 ELISA Pair</td>
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<tr>
<td>554021</td>
<td>HRP Goat Anti-Rabbit IgG</td>
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<tr>
<td>550338</td>
<td>Biotin Goat Anti-Rabbit IgG</td>
<td>1 mL</td>
<td>Polyclonal</td>
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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

- Dai C, Krantz SB. Interferon gamma induces upregulation and activation of caspases 1, 3, and 8 to produce apoptosis in human erythroid progenitor cells. *Blood*. 1999; 93(10):3309-3316. (Biology)