Technical Data Sheet

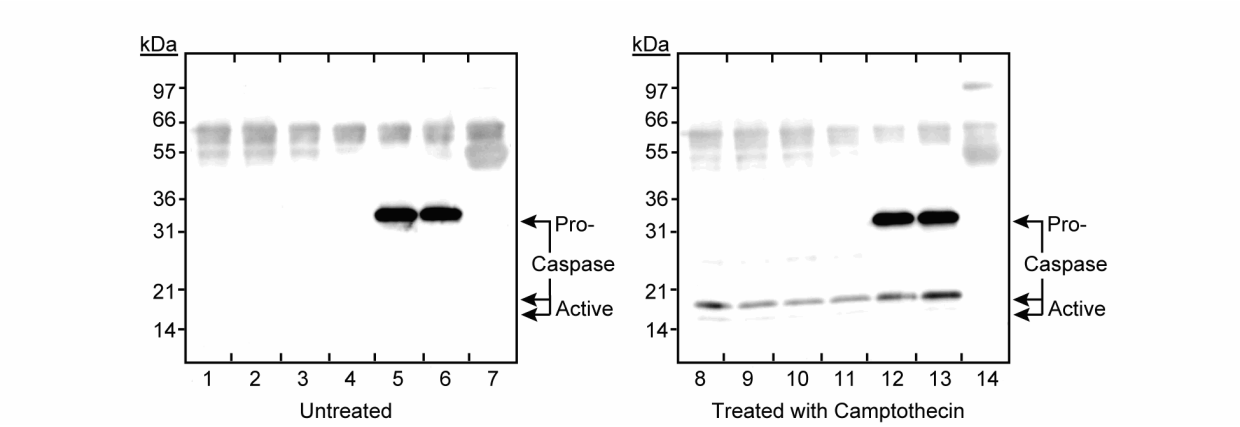
Purified Rabbit Anti- Active Caspase-3

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 (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). Bands may be observable at ~25 kD and ~55-60 kD which represent the light and heavy chains, respectively, of IgG used for the immunoprecipitation.*

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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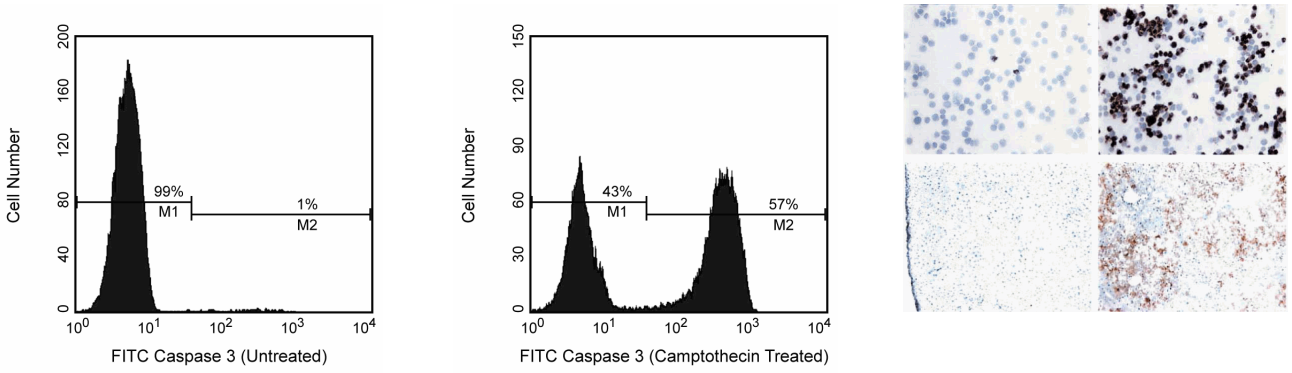

Left: Flow cytometric analysis for active caspase-3. *Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were left untreated (left panel) or treated with 4 µM of camptothecin for 5 hr to induce apoptosis (right panel). Cells were fixed and permeabilized using the BD Cytofix/Cytoperm™ Kit (Cat. No. 554714) 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/1x10e6 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).*

Right: 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

Immunoprecipitation/Western blot	Routinely Tested
Intracellular staining (flow cytometry)	Routinely Tested
ELISA	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging: Please refer to http://www.bdbiosciences.com/support/resources/bioimaging/index.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554254	Purified NA/LE Hamster Anti-Mouse CD95	0.5 mg	Jo2
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
610322	Purified Mouse Anti-Human Caspase-3	50 µg	19/Caspase-3/CPP32
550578	Human Active Caspase-3 ELISA Pair	0.5 ml	(none)
554021	HRP Goat Anti-Rabbit Ig	1.0 ml	(none)
550338	Biotin Goat Anti-Rabbit Ig	1.0 ml	Poly1272

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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.
4. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

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)

Fujita N, Tsuruo T. Involvement of Bcl-2 cleavage in the acceleration of VP-16-induced U937 cell apoptosis. *Biochem Biophys Res Commun.* 1998; 246(2):484-488. (Biology)

Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science.* 1998; 281(5381):1312-1316. (Biology)

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