

## Technical Data Sheet

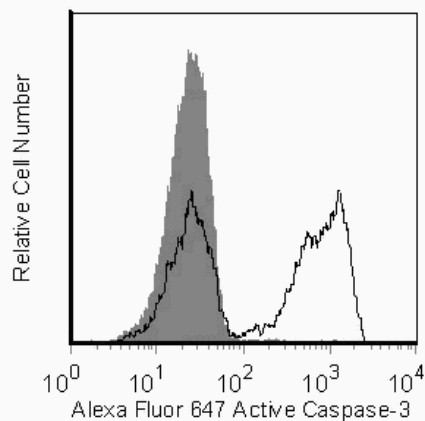
## Alexa Fluor® 647 Rabbit Anti-Active Caspase-3

## Product Information

Material Number:	560626
Alternate Name:	CPP32; Yama; Apopain
Size:	50 tests
Vol. per Test:	5 µl
Clone:	C92-605
Immunogen:	Human Active Caspase-3 Fragment
Isotype:	Rabbit IgG
Reactivity:	QC Testing: Human Predicted: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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.

**Flow cytometric analysis of apoptotic and non-apoptotic populations for Active Caspase-3.**

Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were left untreated (shaded) or were treated with 4-12 µM of camptothecin (Sigma-Aldrich Cat. No. C9911) for 4-6 hr to induce apoptosis (unshaded). Cells were washed once in 1X PBS, then fixed and permeabilized using the BD Cytotfix/Cytoperm™ Kit (Cat. No. 554714) followed by staining with the Alexa Fluor® 647 Rabbit Anti-Active Caspase-3 antibody. Histograms were derived from gated events based on light scattering characteristics for Jurkat cells. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

## Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

## Application Notes

## Application

Intracellular staining (flow cytometry)	Routinely Tested
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## Suggested Companion Products

Catalog Number	Name	Size	Clone
554714	BD Cytotfix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)

## Product Notices

## BD Biosciences

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1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100- $\mu$ l experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. 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.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
9. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

## References

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