# BV650 Rabbit Anti-Active Caspase-3

## Product Information

**Material Number:** 564096  
**Alternate Name:** CPP32; Yama; Apopain  
**Size:** 100 tests  
**Vol. per Test:** 5 µl  
**Clone:** C92-605  
**Immunogen:** Human Active Caspase-3 Fragment  
**Isotype:** Rabbit IgG  
**Reactivity:** QC Testing: Human  
**Tested in Development:** 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.

The antibody was conjugated to BD Horizon™ BV650 which is part of the BD Horizon Brilliant™ Violet family of dyes. This dye is a tandem fluorochrome of BD Horizon BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (e.g., 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.

Flow cytometric analysis of Active Caspase-3 expressed by apoptotic Jurkat cells. Cells from the human Jurkat (Acute T cell leukemia, ATCC TIB-152) cell line were untreated (dashed line histogram) or treated (solid line histogram) with 4 µM camptothecin for 4 hr to induce apoptosis. The cells were washed once in Dulbecco's PBS, fixed and permeabilized using the BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit (Cat. No. 554714; 20 min at room RT), pelleted and washed with BD Perm/Wash™ Buffer (a component of the kit). The cells were subsequently stained with the BD Horizon™ BV650 Rabbit Anti-Active Caspase-3 antibody (Cat. No. 564096). The cells were then washed, resuspended in BD Perm/Wash™ Buffer, and analyzed using a BD LSR II Flow Cytometer System. The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact Jurkat cells.

## 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 with BD Horizon™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

## Application Notes

### Application

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<thead>
<tr>
<th>Intracellular staining (flow cytometry)</th>
<th>Routinely Tested</th>
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## BD Biosciences

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Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
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<tbody>
<tr>
<td>554714</td>
<td>BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit</td>
<td>250 tests</td>
<td>(none)</td>
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Product Notices

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-µl experimental sample (a test).
2. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Brilliant Violet™ 650 is a trademark of Sirigen.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

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)


