BD PharMingen Technical Data Sheet

FITC-CONJUGATED RABBIT ANTI-ACTIVE CASPASE-3 MONOCLONAL ANTIBODY

PRODUCT INFORMATION

Catalog Number:	**559341** (Was: 68654X)
Size:	100 tests
Clone:	C92-605
Storage Buffer:	Aqueous buffered solution containing 0.2% BSA and 0.09% sodium azide.

BACKGROUND

The caspase family of cysteine proteases plays a key role in apoptosis and inflammation *(reviewed in 1).* Caspase-3 (CPP32, Yama, apopain) 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 proenzyme 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 proenzyme.[2] Active caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cytoplasm, e.g., D4-GDI and Bcl-2[3], and in the nucleus, e.g. PARP.

SPECIFICITY AND PREPARATION

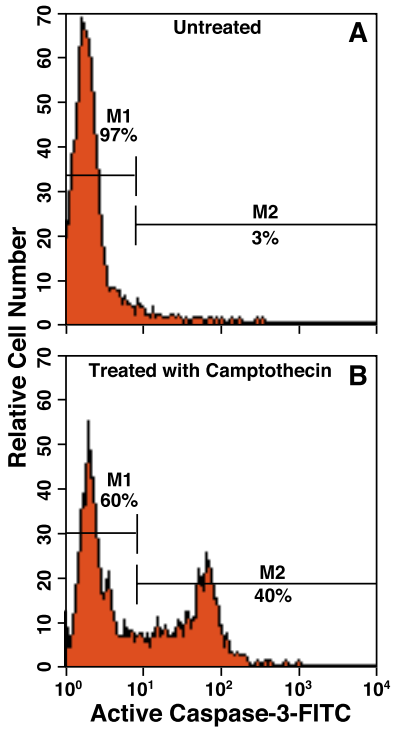
The C92-605 monoclonal antibody (mAb) specifically recognizes the active form of caspase-3 in human and mouse cells. The specificity of the antibody for the active form of caspase-3 was determined by immunoprecipitation experiments; please refer to the technical data sheet for Cat. No. 68651G for further details. The antibody was made against an active human caspase-3 fragment. The antibody is affinity purified and conjugated with FITC under optimal conditions. The solution is free of unconjugated antibody. The antibody is routinely tested by flow cytometry using human Jurkat T cells.

USAGE AND STORAGE

Flow cytometric analysis is the primary application for this antibody. The FITC-conjugated format (Cat. No. 68654X/559341) has been pre-titrated for use at 20 µl/one million cells in flow cytometry. We typically use Jurkat T cells (ATCC TIB-152) which have been treated with Camptothecin to induce apoptosis (see figure legend) as a positive control for this application; however other cell types or methods for induction of apoptosis may also be used for detection of active caspase-3. BD PharMingen offers a purified (unconjugated) format of these antibodies (Cat. No. 68651G/559565) which is also suitable for flow cytometry, as well as immunoprecipitation and immunohistochemical staining of acetone-fixed, frozen cytospins and tissue sections. Store the antibody at 4°C.

REFERENCES

1. Thornberry, N.A. and Y. Lazebnik. 1998. Caspases: enemies within. *Science* 281:1312-1316.
2. Dai, C. and S.B. Krantz. 1999. Interferon gamma induces upregulation and activation of caspases 1, 3, and 8 to produce apoptosis in human erythroid progenitor cells. *Blood* 93:3309-3316.
3. Fujita, N. and T. Tsuruo. 1998. Involvement of Bcl-2 cleavage in the acceleration of VP-16-induced U937 cell apoptosis. *Biochem. Biophys. Res. Commun*. 246:484-488.



Flow cytometric analysis of apoptotic and non-apoptotic populations using the anti-active caspase-3 mAb (clone C92-605). Jurkat T cells were left untreated (A) or treated with 4 µM of camptothecin for 4 hr to induce apoptosis (B). Cells were washed once in PBS, then fixed and permeabilized using the Cytofix/Cytoperm™ Kit (Cat. No. 2075KK) for 20 min at room temperature (RT), pelleted and washed with Perm/Wash™ Buffer (component of Cat. No. 2075KK). Cells were then stained with anti-active caspase-3 mAb (Cat. No. 68654X) using 20 µl/1x10^6 cells for 60 min at RT in the dark. Following incubation with the Ab, cells were washed in Perm/Wash™ Buffer, resuspended in Perm/Wash™ Buffer and analyzed by flow cytometry. The results show that untreated cells were primarily negative for active caspase-3 (A, M1); whereas almost half of the treated cells were positive for active caspase-3 staining (B, M2).

BD Biosciences www.bdbiosciences.com

Clontech | Immunocytometry Systems | Transduction Laboratories
Discovery Labware | PharMingen

For research use only. Not for use in diagnostic or therapeutic procedures. Not for Resale.

Asia Pacific Tel (65) 8610 633 Fax (65) 8601 590
Brazil Tel (55) 11 5185 9833 Fax (55) 11 5185 9834
Canada Tel 888.259.0187 Fax 905.542.9391 canada@bd.com
Europe BD Biosciences Tel (32) 53 720 211 Fax (32) 53 720 450
Europe Life Science Research Tel (49) 6221 305 521 Fax (49) 6221 305 531
Japan Tel (81) 3 5413 8251 Fax (81) 3 5413 8155
Mexico Tel (525) 237 1200 Fax (525) 237 1288
United States Tel 877.232.8995 Fax 858.812.8888

BD Biosciences