Technical Data Sheet

FITC Active Caspase-3 Apoptosis Kit

Product Information

Material Number:	**550480**
Reactivity:	QC Testing: Human Tested in Development: Mouse
Component:	**51-68654X**
Description:	FITC Rabbit Anti- Active Caspase-3 (CPP32; Yama; Apopain)
Size:	100 Tests (1 ea)
Vol. per Test:	20 µl
Clone Name:	C92-605
Immunogen:	Human Active Caspase-3 Fragment
Isotype:	Rabbit IgG
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-6896KC**
Description:	Cytofix/Cytoperm™ Fixation and Permeabilization Solution (1X)
Size:	65 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing paraformaldehyde and saponin.
Component:	**51-6897KC**
Description:	Perm/Wash™ Buffer (10X Solution)
Size:	65 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing saponin, fetal bovine serum 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.

Preparation and Storage

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Induction of Apoptosis by Camptothecin

Materials

1. Prepare a 1.0 mM stock solution of Camptothecin (Sigma-Aldrich Cat. No. C-9911) in DMSO. Camptothecin, an extract of the Chinese tree *Camptotheca acuminata*, is a potent inhibitor of topoisomerase I, a molecule required for DNA synthesis. Camptothecin has been reported to induce apoptosis in a dose dependent manner *in vitro*.
2. Jurkat cells (Human T-cell leukemia; ATCC TIB-152).

Procedure

1. Add camptothecin (4-6 µM final concentration) to 1x10^6 /ml proliferating Jurkat cells.
2. Incubate the cells for 4 hr at 37°C.

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Active Caspase-3 Staining Protocol

Procedure

1. Determine total amount of experimental samples (tests) and calculate the amount of BD Perm/Wash™ buffer (1X) and antibody you will need so that each test will have 100 µl BD Perm/Wash™ buffer (1X) and 20 µl antibody (see chart).
2. Dilute the needed amount of BD Perm/Wash™ buffer (10X) 1:10 in distilled water prior to use.

Note: Precipitate may be occassionally observable with the BD Perm/Wash™ buffer (10X) which will not effect performance of the buffer. The precipitate may be removed by filtering the 1X solution through a 0.45 µm filter.

3. Wash cells twice with cold 1X PBS, then resuspend cells in BD Cytofix/Cytoperm™ solution at a concentration of 1x10^6 cells/0.5 ml.
4. Incubate cells for 20 min on ice.
5. Pellet cells, aspirate, and discard BD Cytofix/Cytoperm™ solution; wash twice with BD Perm/Wash™ buffer (1X) at a volume of 0.5 ml buffer/1x10e6 cells at room temperature.
6. Resuspend cells in the above calculated BD Perm/Wash™ buffer (1X) plus antibody and incubate for 30 min at room temperature.
7. Wash each test in 1.0 ml BD Perm/Wash™ buffer (1X), then resuspend the test in 0.5 ml BD Perm/Wash™ buffer (1X) and analyze by flow cytometry.

Danger: *Cytofix/Cytoperm Fixation and Permeabilization Solution (1X) (component 51-6896KC) contains 4.2% formaldehyde.*

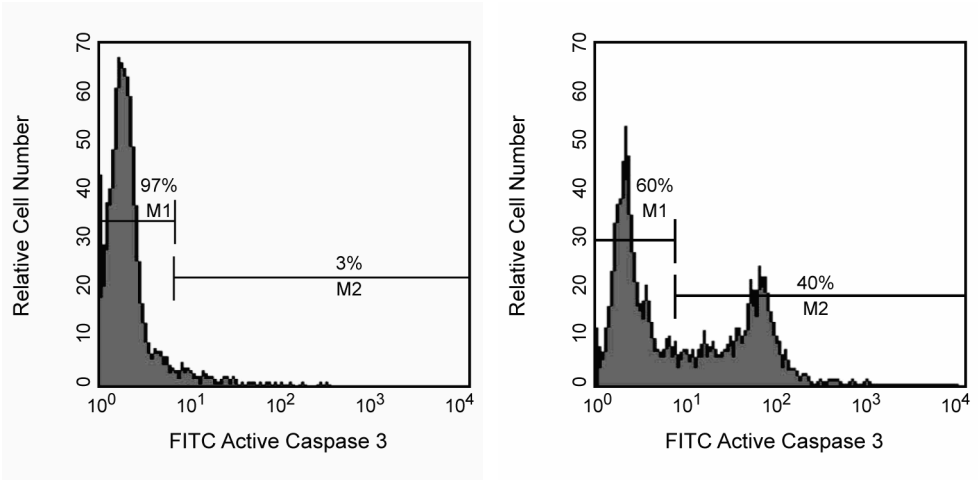
Hazard statements

Harmful if inhaled.
Causes skin irritation.
Causes serious eye damage.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
May cause cancer. Route of exposure: Inhalative.
May cause respiratory irritation.

Precautionary statements

Wear protective clothing / eye protection.
Wear protective gloves.
Do not breathe mist/vapours/spray.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If skin irritation or rash occurs: Get medical advice/attention.

Number of Tests	Number of cells	Perm/Wash™ Volume (ml)	Antibody Volume (µl)
1	$1x10^6$	0.10	20
5	$5x10^6$	0.50	100
10	$10x10^6$	1.00	200
20	$20x10^6$	2.00	400



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 (left panel) or treated for 4 hr with camptothecin (right panel) to induce apoptosis. Cells were permeabilized, fixed, and stained for active caspase-3 as described in the accompanying Staining Protocol. Cells were then analyzed by flow cytometry. Untreated cells were primarily negative for the presence of active caspase-3, whereas greater than one third of the treated cells were positive for active caspase-3 staining (right panel, M2).*

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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 × 10^6 cells in a 100-µl experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Alnemri ES, Livingston DJ, Nicholson DW, et al. Human ICE/CED-3 protease nomenclature. *Cell.* 1996; 87(2):171. (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)

Patel T, Gores GJ, Kaufmann SH. The role of proteases during apoptosis. *FASEB J.* 1996; 10(5):587-597. (Biology)

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