FITC Active Caspase-3 Apoptosis Kit

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.
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 occasionally 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/1x10^6 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.

<table>
<thead>
<tr>
<th>Number of Tests</th>
<th>Number of cells</th>
<th>Perm/Wash™ Volume (ml)</th>
<th>Antibody Volume (µl)</th>
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<tbody>
<tr>
<td>1</td>
<td>1x10^6</td>
<td>0.10</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>5x10^6</td>
<td>0.50</td>
<td>100</td>
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</tr>
<tr>
<td>20</td>
<td>2x10^6</td>
<td>2.00</td>
<td>400</td>
</tr>
</tbody>
</table>

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).
**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. 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.

**References**

