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Datasheet for ABIN2669910

## MitoStep + Apoptosis Detection Kit FITC

### Overview

Quantity:	100 tests
Reactivity:	Chemical
Application:	Flow Cytometry (FACS)

### Product Details

Purpose:	Identify and quantitate apoptotic cells and estimate membrane potential in eukaryotic cells on a single-cell basis by flow cytometry.
Sample Type:	Blood, Cell Culture Cells
Detection Method:	Fluorometric
Characteristics:	Staining cells simultaneously with Annexin V-FITC and the non-vital dye propidium iodide (red fluorescence) allows (bivariate analysis) the discrimination of intact cells (Annexin V-FITC negative, PI negative), early apoptotic (Annexin V-FITC positive, PI negative) and late apoptotic or necrotic cells (Annexin V-FITC positive, PI positive). MitoStep uses a cationic dye DiIC1(5) (1,1',3,3'-hexamethylindodicarbo-cyanine iodide) for the study of mitochondrial $\Delta\Psi$
Components:	<ul style="list-style-type: none"><li>• DiIC1(5), 500 <math>\mu</math>l of 10<math>\mu</math>M in DMSO.</li><li>• Annexin V-FITC provided in liquid form in buffer containing Antibody Stabilizer, PBS, PH 7,4.</li><li>• Propidium Iodide Staining Solution. 100 Tests in PBS (pH 7,4)</li><li>• Annexin V Binding Buffer, 10 X, 45 ml. 0,1M Hepes/NaOH (pH 7,4) 1,4 M NaCl, 25 mM CaCl<sub>2</sub>.</li></ul>
Material not included:	<ul style="list-style-type: none"><li>• pipettes</li><li>,</li><li>• tubes</li><li>,</li><li>• flow cytometry machine</li></ul>

## Target Details

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Molecular Weight: 35kDa

## Application Details

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Application Notes: Optimal working dilution should be determined by the investigator.

Comment: Mitochondrial  $\Delta\Psi$  drives the accumulation in mitochondria of cationic dyes such as cyanines, and the mitochondrial  $\Delta\Psi$  is reduced when energy metabolism is disrupted, notably in apoptosis. Changes in the mitochondrial  $\Delta\Psi$  have been described during necrosis, cell cycle and apoptosis. Mitochondrial uptake of dye is a possible source of fluorescence variance. Samples were tested on Flow Cytometry Samples can be run up to 3 hours after lysis.

Sample Volume: 5  $\mu$ L

Assay Time: < 1 h

Protocol: Staining cells protocol with DiIC1(5), Annexin V and Non-Viable cells solutions (PI or 7-AAD).

1. Prepare Annexin V Binding Buffer: 10 mM Hepes/NaOH (pH 7,4) 140 mM NaCl, 2,5 mM CaCl<sub>2</sub>.
2. Induce apoptosis in cells using the desired method. A negative control should be prepared by untreated cells, that is used to define the basal level of apoptotic and necrotic or dead cells.
3. Harvest the cells after the apoptosis induction.
4. Wash cells twice with temperate PBS and resuspend cells in temperate phosphate-buffered saline (PBS) at a concentration 1 x 10<sup>6</sup> cells/mL.
5. Add 5  $\mu$ L of 10 $\mu$ M DiIC1(5).
6. Incubate the cells at 37 °C, 5 % CO<sub>2</sub>, for 15 minutes.
7. Wash cells twice with temperate PBS and resuspend cells in 1 X Annexin-binding buffer at a concentration 1 x 10<sup>6</sup> cells/mL.
8. Add 5  $\mu$ L of the Annexin V-FITC and 5  $\mu$ L of PI, to each 100  $\mu$ L of cell suspension.
9. Incubate the cells at room temperature for 15 minutes at room temperature (25 °C) in the dark.
10. After incubation period, add 400  $\mu$ L of 1X Annexin-binding buffer.
11. Analyze by flow cytometry within one hour.

Restrictions: For Research Use only

## Handling

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Preservative: Sodium azide

Precaution of Use: Reagents contain sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an

## Handling

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extremely toxic compound. Azide compounds should be diluted with running water before being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Do not pipet by mouth.

Samples should be handled as if capable of transmitting infection. Appropriate disposal methods should be used.

The sample preparation procedure employs a fixative (formaldehyde). Contact is to be avoided with skin or mucous membranes

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Handling Advice:	Light exposure should be avoided. Use dim light during handling, incubation with cells and prior to analysis.
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Storage:	4 °C
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