Datasheet for ABIN2669911

MitoStep + Apoptosis Detection Kit PE

Go to Product page

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 -PE and the non-vital dye propidium iodide (red fluorescence) allows (bivariate analysis) the discrimination of intact cells (Annexin V-PE negative,7-AAD negative), early apoptotic (Annexin V-PE positive, 7-AA-D negative) and late apoptotic or necrotic cells (Annexin V-FITC positive, PI positive).
Components:	• DilC1(5), 500 µl of 10µM in DMSO. • Annexin V-PE is provided in liquid form in buffer containing Antibody Stabilizer, PBS, PH 7,4. • 7-AAD Staining Solution. 100 Tests in PBS (pH 7,4) • Annexin V Binding Buffer, 10 X, 45 ml. 0,1M Hepes/NaOH (pH 7,4) 1,4 M NaCl, 25 mM CaCl2
Material not included:	• pipettes , • tubes , • flow cytometry machine

Target Details

Molecular Weight:	35 kDa

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	Mitochondrial ΔPsi drives the accumulation in mitochondria of cationic dyes such as cyanines, and the mitochondrial ΔPsi is reduced when energy metabolism is disrupted, notably in apoptosis. Changes in the mitochondrial ΔPsi 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 µL
Assay Time:	< 1 h
Protocol:	Staining cells protocol with DilC1(5), Annexin V and Non-Viable cells solutions (PI and 7-AAD). 1. Prepare Annexin V Binding Buffer: 10 mM Hepes/NaOH (pH 7,4) 140 mM NaCl, 2,5 mM CaCl2. 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 106 cells/mL. 5. Add 5 µL of 10µM DilC1(5). 6. Incubate the cells at 37 °C, 5 % CO2, for 15 minutes. 7. Wash cells twice with temperate PBS and resuspend cells in 1 X Annexin-binding buffer at a concentration 1 x 106 cells/mL. 8. Add 5 µL of the Annexin V-PE and 5 µL of 7-AAD, to each 100 µL 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 µL of 1X Annexin-binding buffer. 11. Analyze by flow cytometry within one hour.
Restrictions:	For Research Use only

Handling

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

Handling

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