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# Apoptotic, Necrotic, and Healthy Cells Detection Kit

## Catalog Number: A028

**Table 1. Kit Components and Storage**

| Material | Amount | Storage | Stability |
|---|---|---|---|
| **Andy Fluor 488 Annexin V** (Component A) | **250 µL** | • **2-6 °C,**<br>• **Protect from light** | **The product is stable for 1 year when stored as directed.** |
| **PI** (Component B) | **250 µL** | | |
| **Hoechst 33342** (Component C) | **250 µL** | | |
| **5X Annexin-binding buffer** (Component D) | **25 mL** | | |

**Number of assays:** 50 flow cytometry assays.
**Approximate fluorescence excitation/emission maxima, in nm:** Andy Fluor 488: 495/520; PI: 535/617, bound to DNA; Hoechst 33342: 350/450, bound to DNA.

## Introduction

Apoptosis and necrosis are two processes by which cells die. Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. During apoptosis, phosphatidylserine (PS) is translocated from the inner to the outer surface of the cell, allowing the dying cell to be engulfed by phagocytic cells. Annexin V is a 35 kD $Ca^{2+}$-dependent phospholipid binding protein with a high affinity for PS. The Apoptosis, Necrosis and Healthy Cells Detection Kit features Annexin V labeled with Andy Fluor 488 for staining PS on the surface of apoptotic cells with green fluorescence. Andy Fluor 488 is much brighter and more photostable than traditional green fluorescent dyes like fluorescein.

Necrosis normally results from a severe cellular insult. Both internal organelle and plasma membrane integrity are lost, resulting in spilling of cell contents into the surrounding environment. Propidium iodide (PI) is impermeant to live cells and early apoptotic cells, but stains necrotic cells and late apoptotic cells with red fluorescence.

Apoptosis, Necrosis & Healthy Cell Detection Kit provides a convenient assay for detecting apoptotic (green) and necrotic (red) cells within the same cell population by flow cytometry or fluorescence microscopy. Membrane permeable blue fluorescent nuclear dye Hoechst 33342 is included in the kit for staining the entire cell population.

## Materials Required but Not Provided

- Phosphate buffered saline (PBS)
- Inducing agent
- Deionized water

## Experimental Protocols

### Suspension Cells

**1.** Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

**2.** Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).

**3.** Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 2 mL 5X annexin-binding buffer (Component D) to 8 mL deionized water.

**4.** Re-centrifuge the washed cells (from step 2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer.

Determine the cell density and dilute in 1X annexin-binding buffer to ~1 × $10^6$ cells/mL, preparing a sufficient volume to have 100 µL per assay.

**5.** Add 5 µL Andy Fluor 488 annexin V (Component A), 5 µL PI (Component B) and 5 µL Hoechst33342 (Component C) to each 100 µL of cell suspension.

**6.** Incubate the cells at room temperature for 15 minutes in the dark.

**7.** For flow cytometry analysis, add 400 µL 1X annexin-binding buffer to each tube and analyze fluorescence in DAPI, FITC and PI channels within 1 hour of staining.

**8.** For fluorescence microscopy analysis, wash cells with 1X annexin-binding buffer, resuspend cells in 1X annexin-binding buffer, and observe fluorescence using DAPI, FITC and Texas Red filter sets.

### Adherent Cells

**1.** Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

**2.** After an incubation period, wash the cells twice with cold phosphate-buffered saline (PBS).

**3.** Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 2 mL 5X annexin-binding buffer (Component D) to 8 mL deionized water.

**4.** Prepare staining solution by add 5 µL Andy Fluor 488 annexin V (Component A), 5 µL PI (Component B) and 5 µL Hoechst33342 (Component C) to 100 µL 1X annexin-binding buffer.

**5.** Incubate the cells at room temperature for 15 minutes in the dark.

**6.** Wash cells twice with 1X annexin-binding buffer.

**7.** Cover cells with 1X annexin-binding buffer, and observe fluorescence using DAPI, FITC and Texas Red filter sets.