

# APC Annexin V Apoptosis Detection Kit with 7-AAD

**Catalog Number: KF1012**

## Introduction

The Affinity Biosciences APC Annexin V Apoptosis Detection Kit with 7-AAD contains the reagents needed identify apoptotic or necrotic cells by flow cytometry.

## Kit Components

Components	Size
APC Annexin V	25/100 tests
7-AAD	200 tests
Annexin V Binding Buffer (1X)	50 ml

Affinity Biosciences tests all antibodies by flow cytometry. Citations are provided as a resource for additional applications that have not been validated by Affinity Biosciences. Please choose the appropriate format for each application and consult Materials and Methods sections for additional details about the use of any product in these publications.

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# APC Annexin V

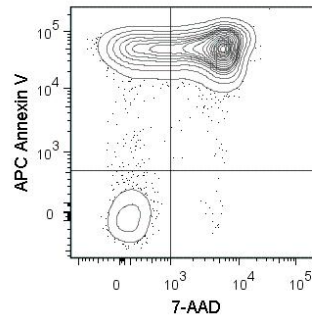
## PRODUCT INFORMATION

**Concentration:** 5µl/test

**Use By:** 12 months from date of receipt

**Storage Conditions:** 2-8°C protected from light

**Formulation:** 10 mM HEPES, 140 mM NaCl, 1mM EDTA, 0.09% NaN<sub>3</sub> pH7.4



Mouse thymocytes were incubated overnight at 37°C in medium containing 1 µM dexamethasone. Cells were harvested and stained with APC Annexin V and 7-AAD Staining Solution.

## DESCRIPTION

Annexin V belongs to the annexin family of calcium-dependent phospholipid binding proteins and has a high affinity for phosphatidylserine (PS). One of the characteristic features of early stage apoptosis is the translocation of PS from the inner to the outer leaflet of the plasma membrane, exposing it to the extracellular environment where it can be detected. Fluorochrome conjugated Annexin V can serve as a sensitive detection probe for this hallmark of early stage apoptotic change.

Annexin V is generally used in combination with a viability dye, such as 7-AAD or propidium iodide (PI), that cannot pass through intact cellular membranes. Translocation of PS occurs prior to loss of membrane integrity, therefore viability dyes would be excluded from the cell. As cells progress through the apoptotic process, membranes are compromised and the viability dyes are able to enter the cell. Using this method, cells that are Annexin V positive and viability dye negative are in early apoptosis. If cells are positive for both Annexin V and the viability dye, they are either undergoing apoptotic death or necrosis. When negative for both markers, cells are viable.

## PREPARATION & STORAGE

Annexin V was conjugated under optimal conditions, with unreacted dye removed from the preparation. It is recommended to store the product undiluted at 4°C, and protected from prolonged exposure to light. Do not freeze.

## APPLICATION NOTES

This Annexin V preparation has been pre-titrated and quality-tested for flow cytometry using mouse thymocytes cultured overnight in the presence of dexamethasone to induce apoptosis. The Annexin V conjugate has been diluted for use at 5 µL per test, defined as the amount of protein that will stain a cell sample in a final volume of approximately 100 µL. The number of cells within a sample should be determined empirically, but typically ranges between 1x10<sup>5</sup> to 1x10<sup>7</sup> cells.

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# 7-AAD

## PRODUCT INFORMATION

**Excitation Laser** Blue (488 nm), Green (532 nm), Yellow-Green (561 nm)

**Emission (nm):** 647

**Formulation:** 10 mM NaH<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl, 0.09% NaN<sub>3</sub>, pH 7.2

**Storage Conditions:** 2-8°C protected from light

**Use by:** 12 months from date of receipt

## DESCRIPTION

7-AAD (7-Aminoactinomycin D) is a nucleic acid dye that can be used to exclude nonviable cells from the analysis of flow cytometry data.

## PREPARATION & STORAGE

7-AAD is provided in solution and should be stored at 2-8°C and protected from light. Do not freeze. CAUTION: 7-AAD is a potential carcinogen. Protect skin and eyes by wearing suitable protective clothing, gloves and eye/face protection.

## APPLICATION NOTES

7-AAD is useful as a viability probe for exclusion of nonviable cells based on light scatter and fluorescence properties. It is recommended to use 5 µL (0.25 µg) of 7-AAD solution per sample and incubate for 5-10 minutes in the dark prior to data acquisition.

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# Annexin V Binding Buffer (1X)

## PRODUCT INFORMATION

**Contents:** Annexin V Binding Buffer (1X)

**Use By:** 6 months from date of receipt

**Storage Conditions:** 2-8°C

**Formulation:** Annexin V Binding Buffer (1X) contains HEPES, NaCl and CaCl<sub>2</sub>.

## DESCRIPTION

Annexin V Binding Buffer is formulated to facilitate the binding of Annexin V conjugates to phosphatidylserine for subsequent analysis by flow cytometry. This buffer is supplied as a 1X solution and requires no manipulation prior to use.

## PREPARATION & STORAGE

Annexin V Binding Buffer (1X) is supplied as a 1X solution and requires no manipulation prior to use.

## APPLICATION NOTES

Annexin V Binding Buffer (1X) is recommended for use with Annexin V reagents. Cells should be resuspended in Annexin V Binding Buffer (1X) prior to incubation with Annexin V reagents.

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