BECKMAN COULTER
MADE IN France

# ANNEXIN A5-FITC KIT-Apoptosis Detection Kit

REF IM3546 200 tests

**For Research Use Only. Not for use in diagnostic procedures.**

## REAGENT CONTENTS

| | Annexin A5-FITC | 10X concentrated Binding Buffer | Propidium Iodide* *Warning: propidium iodide (PI) is toxic |
|---|---|---|---|
| Formulation | Liquid - ready-to-use | Liquid | Red powder |
| Volume | 200 µL | 1.7 mL | 250 µg |
| Number of vials | 1 vial | 6 vials | 1 vial |

### Features of Annexin A5-FITC

Modified human recombinant Annexin labeled with FITC. Displays no measurable anti-coagulant activity in vitro.

- F/P ratio: 1 (stoichiometric complex)
- Purity:>99% pure according to Fast Protein Liquid Chromatography.
- Concentration: 25 µg/mL.

NOTE: Annexin A5 bind optimally to PS at free $Ca^{2+}$ concentration of 1-5 mM or more. Should the medium contain less, adjust concentration by adding $CaCl_2$ or replace the medium by the provided Binding Buffer at 1X concentration. Our experience is that RPMI1640 is less suitable for the assay. Other media such as DMEM may also be used.

## FLUORESCENCE

Annexin A5-FITC:

- Absorption maximum: 492 nm
- Emission maximum: 520 nm

Propidium iodide:

- Absorption maxima: 370 nm, 550 nm
- Emission range: 560 - 680 nm

## APPLICATION

Detection of apoptosis by flow cytometry or fluorescence microscopy.

## WARNING AND PRECAUTIONS

1. This ANNEXIN A5-FITC Kit contains propidium iodide (PI) which is a potential mutagen. We recommend to avoid contact with skin and eyes, to wear suitable protective clothing and gloves, and appropriate eye/face protection. Mechanical ventilation and respiratory protection are also recommended.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Avoid microbial contamination of reagents or incorrect results might occur.
5. Use good laboratory practices when handling these reagents.

## GHS HAZARD CLASSIFICATION

Propidium Iodide

WARNING

| | |
|---|---|
| H315 | Causes skin irritation. |
| H319 | Causes serious eye irritation. |
| H335 | May cause respiratory irritation. |
| P261 | Avoid breathing vapours. |
| P280 | Wear protective gloves, protective clothing and eye/face protection. |
| P304+P340 | IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing. |
| P312 | Call a POISON CENTER or doctor/physician if you feel unwell. |
| P337+P313 | If eye irritation persists: Get medical advice/attention. |

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| P403+P233 | Store in a well-ventilated place. Keep container tightly closed.<br>Propidium iodide >95% |
|---|---|

| SDS | Safety Data Sheet is available at beckman.com/techdocs |
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## STORAGE AND HANDLING CONDITIONS AND STABILITY

These reagents are stable up to the expiration date when stored at 2–8°in the dark. Do not use after the expiration date. Do not freeze.

## REAGENT PREPARATION

- Dilute the 10X concentrated Binding Buffer 10 fold with distilled water and place the diluted buffer on ice. Prepare a quantity sufficient for the expected number of assays.
- Dissolve the 250 µg PI in 1 mL of 1X Binding Buffer and place the PI solution on ice.
- After use, the solutions should be stored at 2 – 8°C.

## PROCEDURE

1. Wash cell samples with ice-cold culture medium or PBS and centrifuge for 5 minutes at 500 x g at 4°C. Discard supernatant, and resuspend the cell pellets in ice-cold 1X Binding Buffer to $5x10^5$~$5x10^6$ cells/mL. Keep tubes on ice.
2. Add 1 µL of annexin A5-FITC solution and 5 µL of dissolved PI to 100 µL of the cell suspensions prepared as given in step 1. Mix gently.
3. Keep tubes on ice and incubate for 15 minutes in the dark.
4. Add 400 µL of ice-cold 1X binding buffer and mix gently.
5. Analyze cell preparations within 30 minutes by flow cytometry (or fluorescence microscopy).

## POSITIVE CONTROLS

1. Incubate cells with 3% formaldehyde-containing PBS for 30 minutes on ice. Centrifuge cells, discard the formaldehyde buffer, and resuspend cell pellets in cold 1X Binding Buffer to $5x10^5$~$5x10^6$ cells/mL. Proceed to staining from step 2 of the staining procedure.
2. Induction of apoptosis of Fas/CD95-expressing cells such as human Jurkat cells or mouse thymocytes.

   Add 100 ng/mL of purified agonistic anti-Fas/CD95 antibody to the culture medium and incubate cells for 4~24 hours at 37°C (5% $CO_2$). Centrifuge cells, discard supernatant, and suspend cell pellets in cold 1X binding buffer to $5x10^5$~$5x10^6$ cells/mL. Proceed to staining from step 2 of the staining procedure.

## GENERAL NOTES AND PRECAUTIONS

The flow cytometer is preferably set such that the distribution of the annexin A5-negative population is in the first decade of the FITC channel and the distribution of the PI-negative population is in the first decade of the PI channel. Optimal parameter settings can be found using a positive control (see above).

The incubation with annexin A5 and PI should be carried out on ice so as to arrest further progress of the cells through the stages of viability ⇒ apoptosis ⇒ secondary necrosis.

For rat thymocytes, when kept on ice, the population distribution (viable, apoptotic, secondary necrotic) remains stable for at least 6 hours.

## SPECIFICITY

The ANNEXIN A5-FITC Kit is an apoptosis detection kit based on the binding properties of annexin A5 to phosphatidylserine (PS) and on the DNA-intercalating capabilities of propidium iodide (PI).

Apoptosis (or programmed cell death) was discovered in tissues on the basis of morphological changes of the cell (1). Gradually, the morphological criteria for apoptosis, like cell shrinkage, nuclear condensation and pyknosis, were complemented with biochemical criteria such as the cleavage of DNA between the nucleosomes, resulting in the ladder appearance of DNA on agarose gels (2). Until recently, this typical feature was considered the hallmark of apoptosis. However, not all cells in apoptosis appear to cleave their DNA strands between the nucleosomes (3) and those that do, do so only late in the apoptotic pathway.

New insights in the apoptotic process led to new parameters, which can be used to detect and measure apoptosis. One of these parameters is the appearance on the surface of the cell of phosphatidylserine (PS), a negatively charged phospholipid usually located in the inner leaflet of the plasma membrane. In the early phase of apoptosis, the integrity of the cell membrane is maintained but the cells lose the asymmetry of their membrane phospholipids (4,5,6,7). PS becomes exposed at the cell surface and forms one of the specific signals for recognition and removal of apoptotic cells by macrophages (5,8).

Annexin A5, a Ca2+-dependent and phospholipid-binding protein, binds preferentially to PS, with high affinity. Apoptotic cell is stained by annexin A5 before the dying cell changes its morphology and hydrolizes its DNA (4,9,10,11,12). The early detection and the ubiquity of apoptosis-associated PS exposure makes the ANNEXIN A5-FITC Kit, in view of its simple and rapid protocol, a powerful tool for the study of apoptosis (9,11,13,14).

In the use of the ANNEXIN A5-FITC Kit, the affinity of annexin A5-FITC for PS in the presence of Ca2+ is exploited. The conjugation of annexin A5 with FITC in a 1:1 stoichiometric complex does not change the native phospholipid-binding properties of Annexin A5. Binding kinetics show a fast association of annexin A5-FITC with the phospholipid membrane, if PS and Ca2+ are available.

The ANNEXIN A5-FITC protocol is designed for the convenient, rapid measure of apoptosis in a sample of suspended cells. Apoptosis-associated PS exposure is a phylogenetically conserved mechanism of mammalian and non-mammalian species, and its detection by annexin A5 has been demonstrated for human, mouse, rat, hamster, chick and drosophila cell types tested so far (5,6,15,8,9,13,16,17,18).

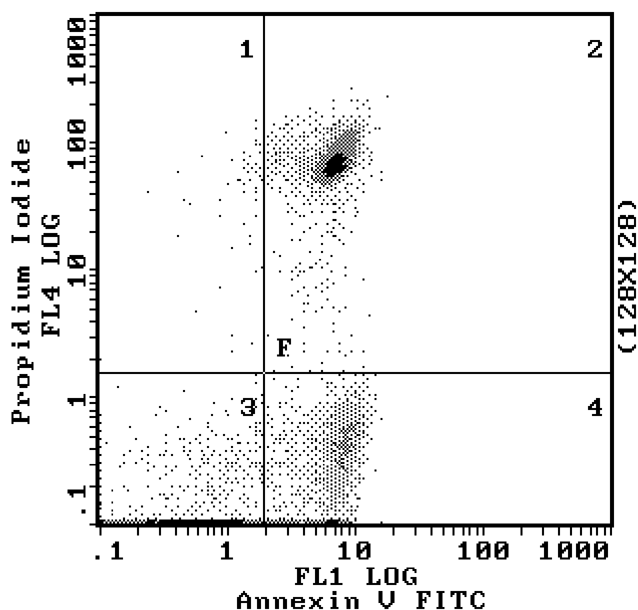
## EXAMPLE DATA

Flow cytometric analysis of apoptotic Jurkat cells after staining by the ANNEXIN A5-FITC kit (performed on a COULTER EPICS XL flow cytometer). Jurkat cells have been treated by 100 ng/mL of agonistic anti-Fas (CD95) antibody for 6 hours. Analysis is done with the XL System II Software. The biparametric representation (FL1 versus FL4) shows three distinct populations, i) the viable cells which have low FITC and a low PI signal, ii) the apoptotic cells, which have high FITC and a low PI signal, iii) the secondary necrotic cells which have high FITC and a high PI signal (see figure). Depending on the cell type and on culture and centrifugation conditions, a fourth population corresponding to the damaged viable cells with low FITC and a high PI signal may be visualized.

Quadrant 2: 40.5% (secondary necrotic cells)

Quadrant 3: 27.1% (viable cells)

Quadrant 4: 31.4% (apoptotic cells)

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## ADDITIONAL INFORMATION

For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-526-7694 (USA or Canada) or contact your local Beckman Coulter Representative.

www.beckmancoulter.com

## Symbols Key

Glossary of Symbols is available at beckman.com/techdocs (document number B60062)

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