7-Aminoactinomycin D (7-AAD)

Catalog #6163

FOR RESEARCH USE ONLY.
Not for use in diagnostic procedures.

INTRODUCTION

7-Aminoactinomycin D (7-AAD, catalog #6163) is an intercalating red fluorescent reagent that binds between cytosine and guanine bases of DNA[1] in membrane-compromised cells. This material, like its parent molecule, Actinomycin D, is a DNA-intercalator with growth-inhibitory properties[1-4]. Normal healthy cells, with intact membrane structure, will exclude the polar 7-AAD vital dye. Mid to late apoptotic and necrotic cells will not exclude this dye and subsequently stain red when 7-AAD complexes with the nuclear DNA. As 7-AAD is membrane impermeant, it cannot reach the DNA in viable cells, thus allowing the identification of cells with permeabilized membranes in a population. 7-AAD distinguishes between living and dead cells by counterstaining nucleic acids red in necrotic, dead, dying, and membrane-compromised cells, while the DNA in healthy cells remains unstained.

7-AAD is supplied as a highly concentrated lyophilized powder at 0.26 mg/vial. Reconstitute it with 260 µL DMSO to yield a stock concentrate at 1 mg/mL. Add it to the cells at a final concentration of 5 µg/mL and analyze with a flow cytometer (Figure 1) or fluorescence microscope (Figure 2). When bound to nucleic acids, the maximum absorption is 546 nm and the maximum emission is 647 nm.

7-aminoactinomycin D may be used in combination with other fluorescent reagents for dual staining or multiplexing purposes. Use it with ICT's FAM-FLICA® poly caspases inhibitor reagent (catalog #637) to include apoptotic cells in the analysis. When used together, these reagents will identify four populations of cells: living; early apoptotic; late apoptotic; and necrotic cells leading to more accurate results when assessing cell death (Figures 2 and 3).

Red fluorescent vital stain for live/dead differentiation

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SPECIFICATIONS

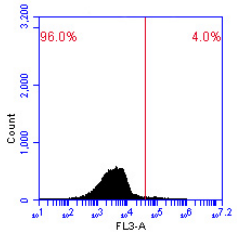
- 0.26 mg/vial
- Lyophilized powder
- CAS number 7240-37-1
- Molecular formula: $C_{62}H_{87}N_{13}O_{16}$
- Molecular weight: 1,270.5 g/mol
- Excitation/Emission = 546/647 nm

SAFETY

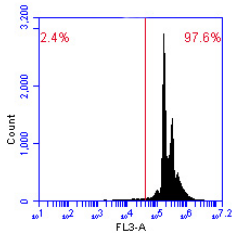
- **DANGER!** Fatal if swallowed. May cause cancer if swallowed. May damage the unborn child if swallowed.
- See Safety Data Sheet (SDS)
- SDS available at www.immunochemistry.com and by calling ICT.

FIGURE 1: LIVE/DEAD CELL STAINING USING 7-AAD

Jurkat cells were grown to 5 x 10^5 cells/mL and split into two populations. One population (A) was left untreated (live) while the other population (B) was treated with 90% ethanol for 60 seconds to create a dead cell population. Cells that were exposed to ethanol (B) were treated with a 5-fold larger volume of PBS to stop the ethanol surface denaturation process. Cells were pelleted by centrifugation (200 x g for 5 minutes) and resuspended in PBS. Cells were then stained with 7-AAD for 10 minutes on ice, and analyzed using an Accuri C6 flow cytometer in FL-3. Only 4% of untreated cells (A) are dead compared with 97.6% of the treated cells (B) (ICT 226:30-31).



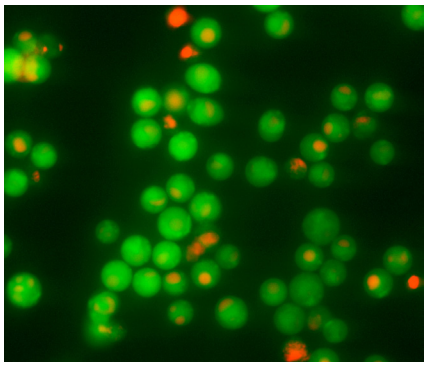
A: Untreated Cells
96% live (left)
4% dead (right)



B: Ethanol-Treated
2.4% live (left)
97.6% dead (right)

FIGURE 2: MICROSCOPY ANALYSIS OF 7-AAD AND FLICA® STAINED APOPTOTIC CELLS

Jurkat cells were exposed to 1 µM of staurosporine for 4 hours at 37°C to induce apoptosis. Cells were dually stained with the green fluorescent FAM-FLICA poly caspase probe to detect apoptosis via caspase activity, and the red fluorescent vital dye 7-AAD to detect necrosis. Early stage apoptotic cells fluoresce green with FAM-FLICA. Dually stained green and red fluorescing cells represent the population of Jurkat cells in mid to late stage apoptosis; these cells have active caspase enzymes and compromised cell membranes. Necrotic cells fluoresce red. Image was obtained using an Olympus BH-2 photomicroscope. FAM-FLICA and 7-AAD were imaged using a 470-490 nm excitation filter plus >520 nm long pass filter tandem (ICT 196:70).



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STORAGE

- ≤ 2-8°C.
- May be stored frozen.
- Protect from light.
- Avoid freeze/thaw cycles.

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HOW TO USE

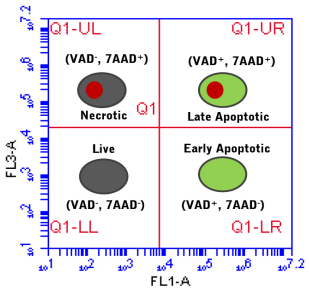
1. Reconstitute the vial of 7-AAD with 0.26 mL DMSO to create a stock concentrate at 1 mg/mL. Mix by swirling or tilting the vial, allowing the DMSO to travel around the base of the amber vial until completely dissolved. At room temperature (RT), the reagent should be dissolved within a few minutes forming a red solution.
2. If storing the stock concentrate for future use, prepare small aliquots (50 µL) to avoid freeze-thaw cycles. The stock concentrate will be stable for 6 months when protected from light and stored at or below -20°C.
3. Expose cells to the experimental conditions.
4. Create 2 control samples:
   - 4a. Untreated viable cells.
   - 4b. Untreated killed cells.
   - For multiparametric flow cytometry analysis, these cells will be stained with 7-AAD to compensate the instrument. These controls will also determine the level of spontaneous cell death that normally occurs within the cell line when compared with the treated cells.
5. Stain cells at a final concentration of 5 µg/mL of 7-AAD in the cell culture. This can be accomplished by:
   - 5a. Pipetting the stock solution directly into the cell suspension at 1:200; e.g., add 2 µL stock to 400 µL cell suspension.
   - 5b. Or by diluting the stock concentrate 1:10 to form the working solution, and then pipetting the working solution into the cells at 1:20. For example, add 50 µL 7-AAD stock concentrate into 450 µL PBS or sterile media. Mix by inverting or vortexing the vial at RT; store on ice up to 2 hours. Then add the working solution to the cell suspension at approximately 1:20; e.g., put 25 µL diluted 7-AAD working solution into 475 µL cell suspension.
6. Incubate 10-30 minutes on ice.
7. If desired, wash cells twice with PBS and fix in 1% paraformaldehyde.
8. Analyze with a fluorescence microscope, fluorescence plate reader, or a flow cytometer. Peak excitation is 546 nm and emission is 647 nm. Dead cells with compromised membranes will appear red (Figures 1-3).

FIGURE 3: MULTIPARAMETRIC ANALYSIS - 7-AAD + FAM-FLICA

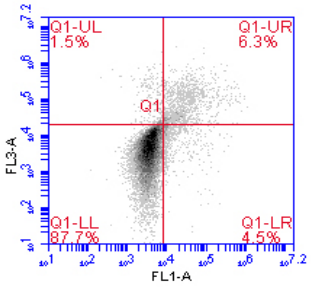
Jurkat cells were treated with a placebo (non-induced treatment with DMSO; A) or treated with 1 µM staurosporine for 4 hours to induce apoptosis via caspase activity (B). Cells were then dually stained with ICT's poly caspase FAM-FLICA apoptosis reagent (Catalog #637) and 7-AAD. Cells were analyzed using an Accuri C6 flow cytometer. Compensation was set using cell populations stained with either FAM-FLICA or 7-AAD (data not shown). FAM-FLICA was analyzed on FL-1, and 7-AAD was analyzed on FL-3. Dual stained density plots (A, non-induced; B, induced populations) are shown.

Flow cytometry can be used to quantitate 4 populations of cells (see Density plot key below). Live, unstained cells do not fluoresce (lower left quadrant). Early stage apoptotic cells fluoresce green with FAM-FLICA (lower right quadrant). Cells in mid to late stage apoptosis will fluoresce both green and red as these cells have active caspase enzymes and compromised cell membranes (upper right quadrant). Necrotic cells fluoresce red (upper left quadrant).

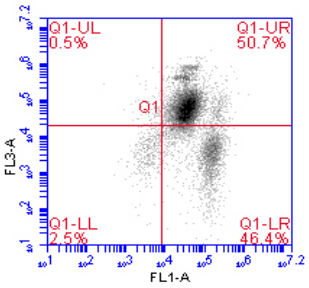
By including FAM-FLICA in the analysis, cells in early apoptosis (lower right quadrant) can be detected, which 7-AAD cannot detect alone. In the non-induced population (A), only 10.8% of cells were apoptotic (LR: 4.5% + UR: 6.3%) compared with 97.1% of the induced population (B; LR: 46.4% + UR: 50.7%). Cells in these quadrants fluoresce green (ICT 226:17-19).



Density plot key



A: Non-Induced



B: Induced

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ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

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