A simple, fast and quantitative single-step dead-cell indicator for flow cytometry

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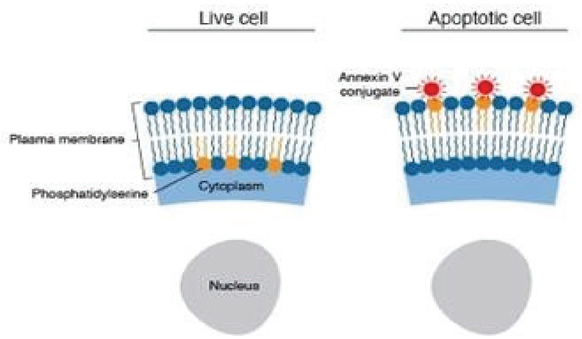
Introduction

The detection and quantification of apoptotic cells has been important in the study of the role of apoptosis in cellular proliferation and differentiation. Combination of DNA stains and highly fluorescent annexin V conjugates provides a quick and reliable detection method for studying apoptosis (Fig.1). DNA binding dye, 7-aminoactinomycin (7-AAD) as shown in Fig. 2 has been used routinely for discrimination of dead cells from viable cells in apoptosis assay. 7-AAD is a fluorescent intercalator with a strong affinity for GC-rich regions of double-stranded DNA. With an absorption maximum at 546 nm, the resulting DNA/7-AAD complexes can be efficiently excited using 543 nm helium-neon laser, 514 nm or 488 nm argon laser lines. Its emission has very large Stokes shift with a maximum in the deep red: 647 nm. In addition, 7-AAD appears to be generally excluded from live cells and can stain dead cells, based on membrane permeability. These properties make 7-AAD widely used in multicolor fluorescence microscopy and flow cytometry. However, 7-AAD has slow penetration rate (30 minute incubation) to cells and poor DNA content histograms in fixed cells due to its large size and poor water solubility. In order to alleviate some of these drawbacks associated with 7-AAD, we have designed and synthesized a wide range of new compounds based on the same fluorescent skeleton as in 7-AAD. The change in structure allows the new compounds to penetrate cells much faster and efficiently. We have evaluated these series of new compounds for dead cell stain and identified a new product, SYTOX® AADvanced™ dead cell stain, which demonstrates improved properties over 7-AAD. After a brief, 5 minute incubation with this new product, the nucleic acid of dead cells fluoresce bright red-orange when excited with 488 nm laser light. Also, the DNA content histogram gives lower CVs than 7-AAD in fixed cells. These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® AADvanced™ dead cell stain a simple, fast and quantitative single-step no-wash dead-cell indicator as well as ideal for use in multicolor application requiring DNA content.

Figure 1. Principle of apoptosis assay using DNA stains and annexin V conjugates



Early apoptosis identified using an annexin V conjugate. In late-stage apoptosis (not shown), the plasma membrane becomes compromised and nucleic acid dyes are permitted to enter the cell and bind with the cell's DNA, which provides an additional marker of apoptosis.

Figure 2. Chemical structure of 7-AAD

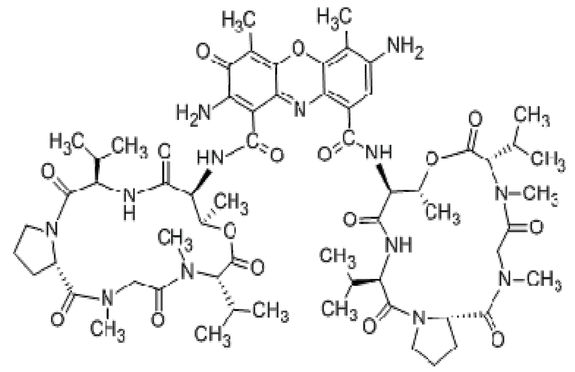
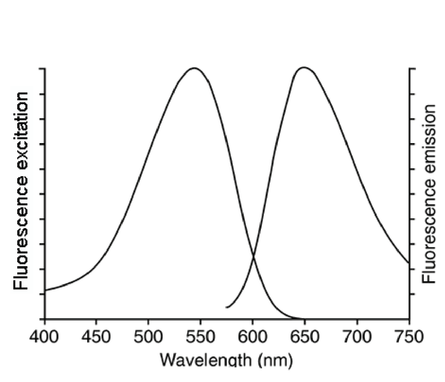
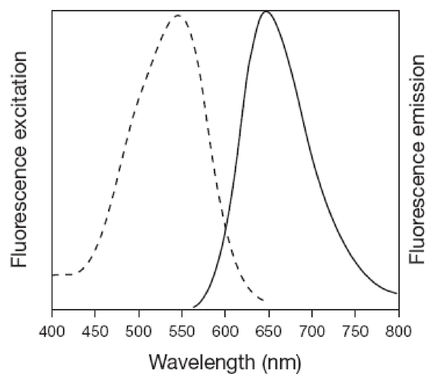


Figure 3. Fluorescence excitation and emission spectra of 7-AAD and the SYTOX® AADvanced ™ dead cell stain bound to DNA (Ab/Em: 546 nm/647 nm)



7-AAD



SYTOX® AADvanced ™ dead cell stain

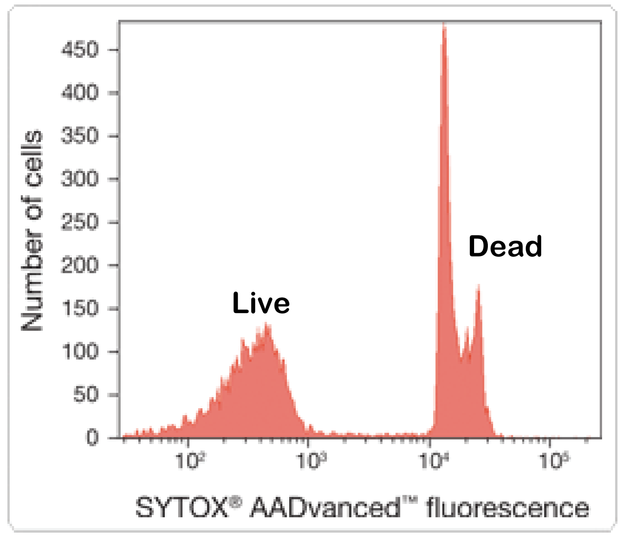


Figure 4. Discrimination of dead cells using the SYTOX® AADvanced™ Dead Cell Stain Kit

A mixture of heat-killed and untreated Jurkat cells were stained with 1 µM of SYTOX® AADvanced™ Dead Cell stain solution for 5 min. Cells were analyzed on a flow cytometer equipped with a 488 nm laser and a 695/40 nm bandpass filter. Live cells are easily distinguished from the dead cell population.

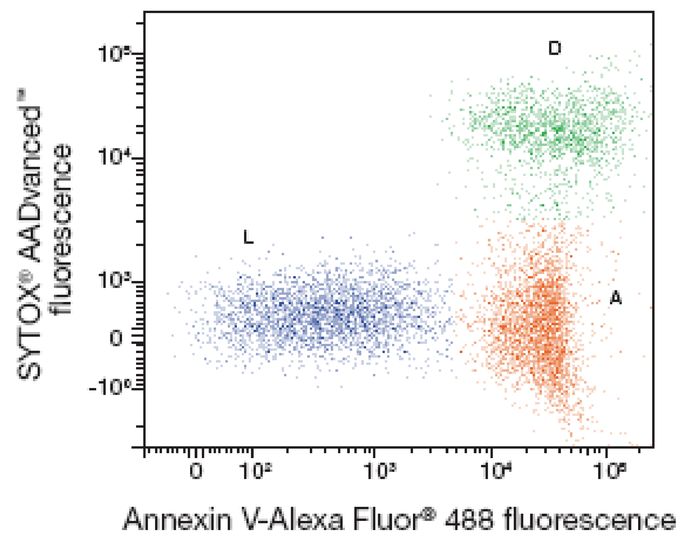


Figure 5. Multicolor application - Staining pattern of Jurkat cells treated with 10 µM camptothecin for 4 hours

Cells were suspended in annexin-binding buffer and then treated with Alexa Fluor® 488 annexin V conjugate for 30 minutes and SYTOX® AADvanced™ dead cell stain for 5 minutes, followed by flow cytometric analysis. Three cell types are identified: live (L), apoptotic (A), and dead (D). SYTOX® AADvanced™ fluorescence was collected in 695/40 bandpass and Alexa Fluor® 488 fluorescence was collected in 530/30 bandpass (both using the 488 nm blue laser).

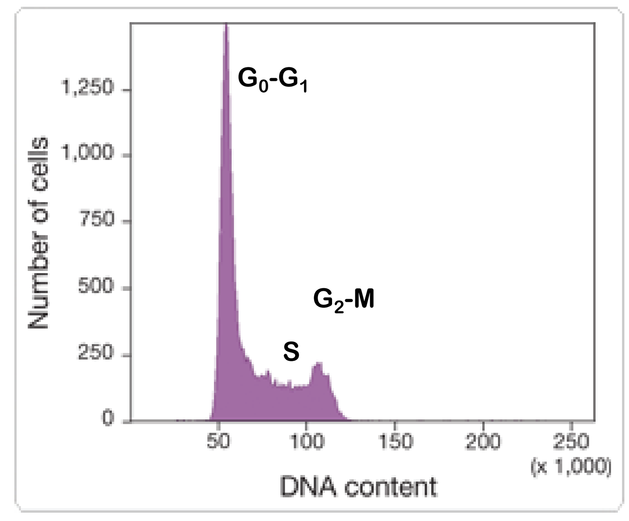


Figure 6. DNA content analysis using the SYTOX® AADvanced™ Dead Cell Stain Kit

HL-60 human promyeloblastic leukemia cells were alcohol fixed and then suspended in 0.1% Triton X/PBS/1% BSA. Cells were stained with 1 µM of SYTOX® AADvanced™ Dead Cell stain solution with the addition of RNase A for 30 min at room temperature. Cells were analyzed on a flow cytometer equipped with a 488 nm laser and a 695/40 nm bandpass filter.

Conclusions

The SYTOX® AADvanced™ Dead Cell Stain Kit offers

- Same spectral properties as 7-AAD - no laser and filter changes for flow cytometer
- Fast labeling of dead cells – only 5 minutes to results
- Efficient cell penetration - better separation of live and dead cells than 7-AAD
- Compatibility with multicolor applications - minimal compensation with the PE channel
- Tighter CVs for more accurate DNA content measurement