

7-AAD Red Fluorescent Live/Dead Stain Reagent

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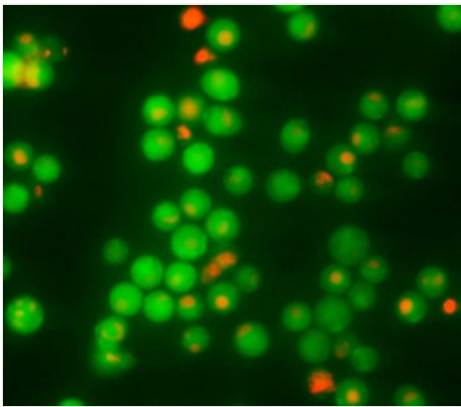
CATALOG #
AAA52740

PRODUCT SYNONYMS

7-AAD Red Fluorescent Live/Dead Stain; 7-AAD Red Fluorescent Live/Dead Stain reagent

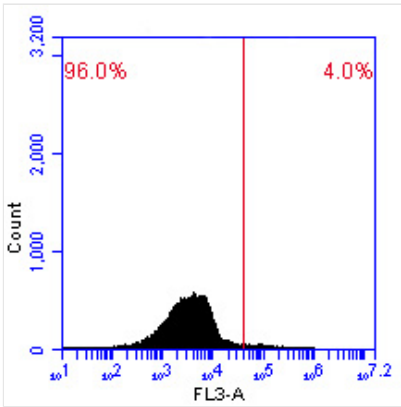
PRODUCT SPECIFICATIONS

Protocol	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, 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 uL) to avoid freeze-thaw cycles. The stock concentrate will be stable for 6 months when protected from light and stored at or below -20 degree C. 3. Expose cells to the experimental conditions. 4. Create 2 control tubes: untreated viable cells and untreated killed cells. These cells will be stained with 7-AAD to compensate the flow cytometer to ensure that dead cells shift along the FL3 axis. 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 ug/mL of 7-AAD in the cell culture. This can be accomplished by pipetting the stock solution directly into the cell suspension at 1:200; e.g., add 2 uL stock to 400 uL cell suspension. This can also be accomplished 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 uL 7-AAD stock concentrate into 450 uL PBS or sterile media. Mix by inverting or vortexing the vial at room temperature. Store on ice up to 2 hours. Then add the working solution to the cell suspension at approximately 1:20; e.g., put 25 uL diluted 7-AAD working solution into 475 uL 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 flow cytometer: excitation at 546 nm; emission at 647 nm in FL3. Dead cells with compromised membranes will appear red.
Excitation/Emission	546 nm / 647 nm
Method of Analysis	Flow cytometer, Fluorescence Microscope
Preparation and Storage	2-8 degree C. May be stored frozen



Application Data

Jurkat cells were exposed to 1 uM of staurosporine for 4 hours at 37 degree 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).



Application Data

Jurkat cells were grown to 5 x 10^5 cells/mL and split into two populations. One population (left) was left untreated (live) while the other population (right) was treated with 90% ethanol for 60 seconds to create a dead cell population. Cells that were exposed to ethanol (right) 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 (left) are dead compared with 97.6% of the treated cells (right) (ICT 226:30-31).

ADDITIONAL INFORMATION

Related Product Information for 7-AAD Red Fluorescent Live/Dead Stain reagent	7-Aminoactinomycin D is an intercalating red fluorescent reagent that binds between cytosine and guanine bases of DNA in membrane-compromised cells. This material, like its parent molecule, Actinomycin D, is a DNA-intercalator with growth-inhibitory properties. 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 the dye 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.
Product Categories/Family for 7-AAD Red Fluorescent Live/Dead Stain reagent	Cell Viability Assays; Cellular Imaging; 7-AAD Red Fluorescent Live/Dead Stain

NCBI AND UNIPROT INFORMATION

Molecular Weight	1,270
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For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/reagent/7-aad-red-fluorescent-live-dead-stain/52740>