Revised: November 5, 2020

Product Information

7-AAD (7-aminoactinomycin D)

Catalog no.	Product	Size
40084	7-AAD in DMSO:water 1:1, 1 mg/mL	1 mL
40037	7-AAD	1 mg

Storage and Handling
Store at -20°C. Protect from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Molecular Information: $C_{62}H_{87}N_{13}O_{16}$
CAS number: 7240-37-1
Molecular Weight: 1270
Color and Form: Red solution (40084); orange/red solid (40037)
Solubility: Soluble in DMSO or DMF
Absorption/Emission: 546/647 nm (with DNA)

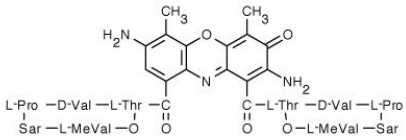

Figure 1. Structure of 7-Aminoactinomycin D.

Product Description
7-AAD is a fluorescent DNA binding dye that is membrane impermeant and therefore generally excluded from viable cells. The dye is excluded by live cells and early apoptotic cells, but stains necrotic and late apoptotic cells with compromised membrane integrity.

7-AAD/DNA complexes can be excited at 488 nm with an argon-ion laser, and have a large Stokes shift with an emission maxima of 647 nm (1-3). 7-AAD intercalates selectively at GC-rich regions of DNA, which makes the dye useful for chromosome banding studies (4). 7-AAD can be used in multicolor fluorescence microscopy and flow cytometry.

References
1) Exp. Parasitol. 97, 141(2001); 2) Br. J. Haematol. 104, 530(1990); 3) Cytometry 12, 221(1991); 4) Chromosoma 68, 287(1978).

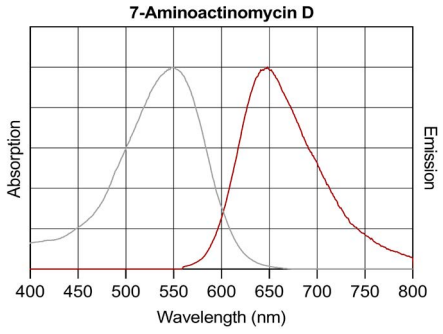

Figure 2. Normalized absorbance and emission spectra with DNA.

General Considerations
We recommend using the dye at 1 ug/mL (1:1000 dilution) for live/dead discrimination or 10 ug/mL (1:100 dilution) for cell cycle profiling. Example protocols are provided below.

Experimental Protocols
For live/dead discrimination by flow cytometry
1. Prepare a positive control by incubating cells at 56°C for 30 minutes then cool to room temperature. Include an untreated cell sample as a negative control.
2. Adjust cells to $5x10^6$ cells per mL in complete culture medium or buffer of your choice and aliquot 1 mL per flow tube.

   Note: Cells can be stained anywhere between $5x10^5$ cells/mL to 10^7 cells per mL in 100 uL to 1 mL. If necessary, a 100 ug/mL intermediate dilution of 7-AAD can be prepared by diluting the stock solution 1:10 in water or buffer.
3. Add 1 uL of 1 mg/mL 7-AAD to 1 mL of cells and mix.
4. Incubate 15-30 minutes at room temperature, protected from light. The incubation can be carried out on ice if desired.
5. Analyze by flow cytometry in the PE-Cy®5 or PerCP channel without washing the cells.

Notes:

a. While 7-AAD staining is retained after formaldehyde fixation, separation between live and dead cells is reduced after fixation due to dye transfer from dead to live cells. For truly fixable dead cell staining, we recommend using a covalent dye such as our Live-or-Dye™ stains (see Related Products).

b. This protocol was optimized using Jurkat cells. Assay optimization may be required for use with other cell types.

c. If you prefer not to wash your cells, staining can be performed in cell culture medium with serum instead of buffer.

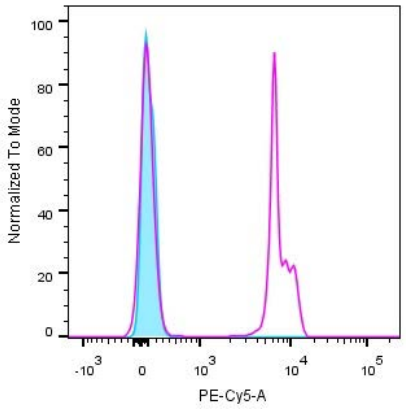

Figure 3. Live/dead discrimination with 7-AAD by flow cytometry analysis. A mixture of live and heat-killed Jurkat cells were left unstained (blue peak), or stained with 1 ug/mL 7-AAD (magenta, open peaks), and analyzed on a BD LSRII flow cytometer. The live cell peak is on the left, with low fluorescence similar to unstained cells, while the fluorescent dead cell peaks are shifted to the right.

For cell cycle profiling by flow cytometry analysis of DNA content

Materials required but not provided (see Related Products)

- Flow Cytometry Fixation/Permeabilization Kit (catalog no. 23006)
- 1X Phosphate buffered saline (PBS) or your preferred FACS buffer

Staining Protocol

1. Adjust cells to 10^7 cells per mL and aliquot 100 uL per flow tube.
2. Fix and permeabilize cells according the protocol for the Flow Cytometry Fixation/Permeabilization Kit, or use your preferred method.
3. Pellet the cells by centrifugation and wash with 1X PBS or FACS buffer.
4. Pellet the cells by centrifugation and resuspend in 100 uL buffer.
5. Add 1 uL of 1 mg/mL 7-AAD per tube and mix by gentle vortexing.
6. Incubate 15 minutes at room temperature, protected from light.
7. Add 400 uL PBS or FACS buffer per tube. Analyze by flow cytometry in the PE-Cy®5 channel or PerCP channel. Use a linear scale for fluorescence detection, and acquire data with a slow flow rate (~12 uL /minute).

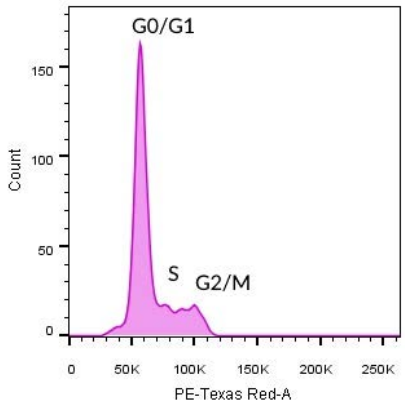

Figure 4. Cell cycle profiling by flow cytometry using 7-AAD for DNA content analysis. Jurkat cells were fixed with ice-cold methanol, then stained with 10 ug/mL 7-AAD in PBS for 15 minutes. Cells were analyzed on a BD LSRII flow cytometer in the PE-Texas Red® channel on linear scale.

Related Products

Catalog number	Product
40085	NucSpot® Far-Red Nuclear Stain
40083	NucSpot® 470 Nuclear Stain
32010	Live-or-Dye NucFix™ Red Staining Kit
40081	NucSpot® Live 488
40082	NucSpot® Live 650
30060	CF®488A Annexin V and 7-AAD Apoptosis kit
30061	CF®488A Annexin V and PI Apoptosis kit
40016	Propidium Iodide
40091	Oxazole Blue
40089	Oxazole Yellow
40061	RedDot™2 Far Red Nuclear Stain
40087	Thiazole Red
40086	Thiazole Green
40080	Thiazole Red Homodimer
40010	Ethidium Homodimer I
40050	Ethidium Homodimer III
40011	DAPI
40045	Hoechst 33258
40047	Hoechst 33342
23066	Flow Cytometry Fixation/Permeabilization Kit
90082	DMSO, Anhydrous

Please visit our website at www.biotium.com for information on our life science research products, including Live-or-Dye™ cell viability kits, cellular stains, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.