7-aminoactinomycin D

Cat. No. 7-AAD-024 **Lot. No.** (See product label)

SPECIFICATION

Description	7-AAD (7-aminoactinomycin D) is a membrane-impermeant fluorescent DNA binding dye that is useful for live/dead discrimination and cell cycle profiling by flow cytometry. 7-AAD is a fluorescent DNA binding dye that is membrane impermeant and therefore generally excluded from live cells and early apoptotic cells, but stains necrotic and late apoptotic cells with compromised membrane integrity.
Form	Orange/red solid
Molecular Mass	1270.45
Molecular Information	C62H87N13O16
CAS number	7240-37-1
Solubility	Soluble in DMSO or DMF
Absorption/Emission	546/647 nm (with DNA)
General Staining Protocol	For live/dead discrimination by flow cytometry 1. Prepare a positive control by incubating cells at 56 centigrade for 30 minutes then cool to room temperature. Include an untreated cell sample as a negative control. 2. Adjust cells to 5×10^6 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 5×10^5 cells/mL to 10^7 cells per mL

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in 100 µL to 1 mL. If necessary, a 100 µg/mL intermediate dilution of 7-AAD can be prepared by diluting the stock solution 1:10 in water or buffer.

3. Add 1 µL 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.

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.

For cell cycle profiling by flow cytometry analysis of DNA content

Materials required but not provided

• Flow Cytometry Fixation/Permeabilization Kit

• 1× Phosphate buffered saline (PBS) or your preferred FACS buffer

Staining Protocol

1. Adjust cells to 10^7 cells per mL and aliquot 100 µL 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 µL buffer.

5. Add 1 µL 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 µL 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 µL /minute).
Probe cellular localization	Nucleus
Detection method/readout	Flow cytometry
Assay type/options	DNA content/cell cycle profiling (flow cytometry), Live/dead discrimination, No-wash staining
Cell permeability	Membrane impermeant
Apoptosis/viability marker	Dead cell stain
Colors	Far-red
Applications	We recommend using the dye at 1 µg/mL (1:1000 dilution) for live/dead discrimination or 10 µg/mL (1:100 dilution) for cell cycle profiling.
Stability	Product is stable for at least 12 months from date of receipt when stored as recommended.
Storage	Store at -20 centigrade. Protect from light.
Reference	1. Exp. Parasitol. 97, 141(2001). 2. Br. J. Haematol. 104, 530(1990). 3. Cytometry 12, 221(1991). 4. Chromosoma 68, 287(1978).

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