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Product Information

7-AAD (7-Aminoactinomycin D)

Catalog Number	Unit Size
C012	1 mg

Storage upon receipt:
- -20°C
- Protect from light

Ex/Em: 546/647 nm, bound to DNA

Product Description

7-Aminoactinomycin D (7-AAD) is a membrane-impermeant fluorescent DNA binding dye that is useful for live/dead discrimination and cell cycle analysis by flow cytometry. 7-AAD/DNA complexes can be excited by the 488 nm laser and has an emission maxima of 647 nm, making this nucleic acid stain useful for multicolor fluorescence microscopy and flow cytometry. 7-AAD appears to be generally excluded from live cells and early apoptotic cells, but stains necrotic and late apoptotic cells with compromised membrane integrity. 7-AAD has been used for cell cycle analysis by flow cytometry. 7-AAD also intercalates selectively at GC-rich regions of DNA yielding a distinct banding pattern in polytene chromosomes and chromatin for use in chromosome banding studies.

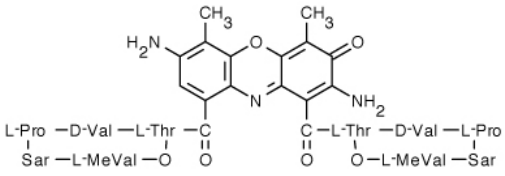
Specifications

Chemical Name: 7-Aminoactinomycin D

Molecular Formula: $C_{62}H_{87}N_{13}O_{16}$

Molecular Weight: 1270.45

CAS Number: 7240-37-1



General Considerations

We recommend using the dye at 1 µg/mL for live/dead discrimination or 10 µg/mL for cell cycle analysis.

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 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 in 100 µL to 1 mL.
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.

For cell cycle analysis by flow cytometry

1. Adjust cells to 10^7 cells per mL and aliquot 100 µL per flow tube.
2. Fix and permeabilize cells according to the protocol for the Flow Cytometry Fixation/ Permeabilization Kit, or use your preferred method.
3. Pellet the cells by centrifugation and wash with 1× 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 fluoresceence detection, and acquire data with a slow flow rate (~12 µL /minute).