7-AMINO-ACTINOMYCIN D STAINING OF DEAD CELLS FOR FLOW CYTOMETRY

7-Amino-actinomycin D (7-AAD) intercalates into double-stranded nucleic acids. It is excluded by viable cells but can penetrate cell membranes of dying or dead cells.

I. MATERIALS:

A. 7-Amino-actinomycin D (e.g., Cat #129935, EMD Millipore, MA)

B. Absolute methanol or DMSO

B. 1 X PBS with Ca2+ and Mg2+

C. Buffer: PBS (Ca2+ and Mg2+ free)
+2% newborn calf serum (or 0.2% BSA)
+0.1% sodium azide

7-AAD stock buffer:

For long-term storage, store unopened vials of 7-AAD in the freezer. Dissolve 1 mg of 7-AAD powder by adding 50 µL of absolute methanol (or DMSO) directly to the vial. Mix well and add 950 µL of 1 X PBS with Ca2+ and Mg2+ to achieve a concentration of 1 mg/mL. Store solution tightly closed and protected from light at 4°C. We have kept this solution for several months and have not observed loss in staining activity.

II. METHOD:

Stain your cells as outlined in the protocol for single color or dual-color staining with FITC-, PE-, and other fluorochrome-labeled monoclonal antibodies.

After the last washing step resuspend your cells as usual in 0.5 mL of buffer for analysis. If you want to assess viability of your samples add 0.5-1 µL of the 7-AAD stock solution to each tube and mix well. Keep the samples in this solution at 4°C protected from light for approximately 20 minutes or until analysis on the flow cytometer.

7-AAD can be used for dead cell exclusion on samples that are stained with PE (phycoerythrin)-conjugated antibodies as well as others, because the emission of 7-AAD can be easily compensated out of other fluorescent channels on the flow cytometer.

Ref.: Schmid I, Uittenbogaart CH, Krall WJ, Braun J and Giorgi JV. Dead cell discrimination with 7-amino-actinomycin D in combination with dual color immunofluorescence in single laser flow cytometry. Cytometry 13:204-208, 1992.