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, Calbiochem, San Diego, CA)

B. 1 X PBS with Ca^{2+} and Mg^{2+}

C. Buffer: PBS (Ca^{2+} and Mg^{2+} 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 microliters of absolute methanol directly to the vial. Mix well and add 950 microliters of 1 X PBS with Ca^{2+} and Mg^{2+} 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 and/or PE-labeled monoclonal antibodies.

After the last washing step resuspend your cells as usual in 1 ml of buffer for analysis. If you want to assess viability of your samples add 1 m l 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.

***NOTE**:* This method can now be used in combination with formaldehyde fixation of samples. Samples are first stained with 7-AAD, then fixed in 1% formaldehyde that contains 2-5 microliters/ml of actinomycin D (ref. Fetterhoff et al.); see attached protocol. 7-AAD can be used for dead cell exclusion on samples that are stained with PE (phycoerythrin)-conjugated antibodies, because the emission spectra of 7-AAD and PE can be easily separated 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.

Fetterhoff TJ, Holland SP, and Wile, KJ. Fluorescent detection of non-viable cells in fixed cell preparations. Cytometry 14 (Suppl. 6):27, 1993.

Protocol for the use of actinomycin D (AD) on 7-AAD stained, formaldehyde-fixed samples

I. Materials:

A. Actinomycin D (C_1) (AD, e.g., Cat# 102008, Boehringer Mannheim, IN)

B. 1 X PBS

C. Sonicator

D. Formaldehyde solution (see protocol for preparation of 2% stock solution)

II. Preparation of AD stock solution (1mg/ml):

To 1mg of AD powder add:

50 microliters of ice-cold absolute ETOH, vortex

950 microliters of 1 X PBS

Sonicate the resulting solution for 10 min at 4°C; keep the solution overnight in the refrigerator at 4°C, protected from light before using it.

Store solution at 4°C protected from light.

Working dilution is 2-5 micrograms/ml.

III. Method:

Cells are first incubated with 7-AAD for approximately 20 min, spun down and washed once with 1 X PBS. Then, a 1% formaldehyde solution containing 2-5 microliters/ml of AD (F/AD) is quickly added to the cell pellet. Cells have to be stored in the cold protected from light and can be analyzed approximately 30 min after the addition of the F/AD solution. Cells are run on the flow cytometer in the F/AD solution. We have stored samples up to 3 days without any loss in the ability to discriminate dead from live cells.