

## Protocol 609\_Exclusion of dead cells in FACS staining using 7-AAD (7-AAD: Sigma A9400-1MG)

### General

7-Amino-actinomycin D (7-AAD) is a fluorescent chemical compound with a strong affinity for DNA. It is used as a fluorescent marker for DNA in fluorescence microscopy and flow cytometry. It intercalates in double-stranded DNA, with a high affinity for GC-rich regions.

With an absorption maximum at 546 nm, 7-AAD is efficiently excited using a 543 nm helium-neon laser; it can also be excited with somewhat lower efficiency using the 488 nm or 514 nm argon laser lines.

7-AAD does not readily pass through intact cell membranes; 7-AAD is used as a cell viability stain. Cells with compromised membranes will stain with 7-AAD, while live cells with intact cell membranes will remain dark.

### Specific

- On a FACSCalibur, 7-AAD staining is detected in the FL3 channel. Therefore you cannot use PerCp-conjugated antibodies for these samples (or other fluorophores that are detected in FL3).
- **DO NOT FIX YOUR SAMPLES!!!**
- The 7-AAD stock vial is located in the "FACS antibody stock box" in the FACS antibody fridge in 3N11B. The stock concentration is 1mg/ml. Final concentration for staining of dead cells will be 0.5-1µg/ml.
- To make up a new stock vial, dissolve 1mg of 7-AAD in 50µl absolute methanol (3N42) and add 950µl PBS.
- Open the 7-AAD vial **ONLY** in the sterile hood, because it is used for staining of samples for FACS sorting.

### Procedure

- As for every FACS staining, you need single stains for every color. Therefore you must include a single stain for 7-ADD for compensation.

**Important: Make sure that your unstained sample and your single stains (except the 7-AAD single stain) DO NOT get treated with 7-ADD!**

- After your last wash step, re-suspend cells in 400µl FACS buffer.
- Dilute 7-AAD 10x in FACS buffer. You need 0.4µl of stock 7-AAD per 400µl sample. E.g. if you have 30 samples, dilute 12µl 7-ADD (1mg/ml) in 108µl (=120-12) FACS buffer.
- Add 4µl of the diluted 7-ADD to your sample (400µl) and mix well.
- **TIPP:** To make pipetting easier, dilute 7-ADD again 10x in FACS buffer (e.g. add 1200-120=1080µl FACS buffer to the 120µl 7-ADD solution) and add 40µl per 400µl sample.
- If your sample is more than 400µl (e.g. for FACS sorting) adjust the amount of 7-AAD accordingly.
- Most protocols recommend staining for 5-20min. However, for simple dead cell exclusion, 7-AAD can be added just before acquiring the samples on the FACSCalibur.
- **DO NOT** add 7-AAD hours before acquiring!