

NOTE:

PROCEDURE FOR CELL COUNTS AND VIABILITIES USING ACRIDINE  
ORANGE/ETHIDIUM BROMIDE (AO/EB) OR FLUORESCEIN DIACETATE (FDA)

NOTE:

This procedure should be used when accurate viabilities or viable cell counts are important.

AO/EB

1. The mixture consists of 1 PPM (10-4%) each of AO and EB in PBS. .
2. Suspend the cells at a normal counting concentration (about  $1-5 \times 10^9$  fml).
3. Mix cells and AO/EB mixture 1:1 (e.g., 50ul of each).
4. Add a sample of this mixture to a hemocytometer slide.
5. On the American Optical microscope turn on the tungsten-halogen lamp to 9V and the normal white light to 4.5V.
6. Place the slide on the microscope stage and use the white light to find the counting grid and to focus. The white light is controlled by the two small handles in the lamp housing.
7. Using the 20X objective, turn down the white light until both the grid and the cell fluorescence are visible. If no fluorescence is visible check the handle above the microscope objective area; it should be in the right position (to the right), and blue light should be coming from the objective.
8. You should see:
  - live cells - green
  - dead cells - orange nucleus
  - erythrocytes - unstained
  - non-nucleated cell culture blebs - unstained to faint orange  
like dead cell cytoplasm

Count the types of-interest. (If more fluorescence brightness is needed, turn up the lamp to 12V. This should not be done unnecessarily since bulb life is about 10 times longer at 9V.)

9. Turn off the tungsten-halogen lamp and white light unless someone wants to use the microscope immediately.

NOTE: EB and AD are nucleic acid stains and are possibly carcinogenic. The stock solution is supplied at high dilution but handle it carefully and clean up spills. ES and AD stick to plastic tubing, so cells stained with them require special precautions in use of the FACS.

Stock AO .1% => dilute 1/1000 } staining solution use 1:1  
EO .1% => dilute 1/1000