

# VWR® Automated Cell Counter Fluo – AO/PI Staining

## Acridine Orange/Propidium Iodide Live & Dead Cell Co-staining

### **Assay Principle:**

Acridine Orange (AO) is a cell-permeant DNA/RNA binding dye that stains all cells with green fluorescence. Propidium Iodide (PI) is a membrane-impermeant DNA/RNA binding dye that selectively stains dead cells with damaged membranes with red fluorescence.

### **Materials:**

- VWR® automated cell counter Fluo. Cat. No. 49893-2000
- VWR® cell counting slide (2 samples/slide). Cat. No. 10228-0050
- VWR® Fluo cube for GFP and AO, green. Cat. No. 49893-4951
- VWR® Fluo cube for PI, red. Cat. No. 49893-4952
- Acridine Orange, 10 mg/mL in Water. EU Cat. No. BTIU40039 ; NA Cat.No. 89139-110
- Propidium Iodide, 50 ug/mL in Buffer. EU Cat. No. BTIU40048 ; NA Cat. No. 89411-112

### **Procedure:**

Note: Cells may be stained in culture medium without washing.

1. Prepare 2X staining solution as follows:
  - a. Prepare an intermediate dilution of 1 mg/mL Acridine Orange (AO) by combining 1 uL of 10 mg/mL AO and 9 uL of PBS. Pipette up and down to mix well.
  - b. Add 1 uL of the 1 mg/mL AO solution from the previous step to 100 uL of 50 ug/mL Propidium Iodide (PI). Vortex to mix well.
2. Mix 20 uL of cells with 20 uL of 2X staining solution from step 1b. Pipette up and down gently to mix. The final concentration of AO will be 5 ug/mL and the final concentration of PI will be 25 ug/mL.
3. Incubate 5 minutes at RT.
4. Mix the cells again by gently pipetting up and down, and then load 10 uL to the counting slide for analysis in the BF, AO, and PI channels.