Evaluation of Cellular Viability with Propidium Iodide or 7-Amino-Actinomycin D

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Introduction
The enumeration and discrimination of living and dead cells can be determined with an evaluation of Cellular Viability. Living cells have intact cell membranes and active cell metabolisms that exclude Propidium Iodide (PI) and 7-Amino-Actinomycin D (7-AAD) viability dyes, while cells with damaged membranes or impaired metabolisms allow these dyes to enter the cell. Cells that actively uptake these dyes will fluoresce brightly in the red range of the visible color spectrum with wavelengths of 550 nm to approximately 720 nm.

Viability determinations are important in Cell Culture, Cryopreservation, and Flow Cytometry and have been used in applications that assess substrate and drug cytotoxicity, cytotoxic cellular interactions and as a method for eliminating non-viable cells in immunofluorescence assays.

The Cell Lab Quanta™ is a flow cytometry system designed to simultaneously measure Electronic Cell Volume (EV) and fluorescence. EV and, either PI or 7-AAD, provide for a rapid and reliable method of discriminating living and dead cell populations. EV also helps to discriminate clumps of cells, small dying cells, and debris.

In this Application Note, a method for viability is described that is simple and accurate using Cell Lab Quanta.

Materials and Methods
- Flow-Check™ Fluorospheres PN 6605359
- PI (DNA Prep Stain) PN 6607055
- 7-AAD PN IM3422
- Cell concentrations of $1 \times 10^6$ cells/mL

Method for Propidium Iodide:
1. Blank - Add 1 mL of cells to a sample cup.
2. Viability - Add 0.9 mL of cells and 0.1 mL viability dye to a sample cup. Vortex.
3. Analyze within 5 - 10 minutes

Method for 7-AAD:
1. Blank - Add 1 mL of cells to a sample cup.
2. Viability - Add 1.0 mL of cells and 20 µL viability dye to a sample cup. Vortex.
3. Analyze within 5 - 10 minutes

Perform instrument alignment with Flow-Check Fluorospheres.

Default Protocol:
Trigger on EV in the first histogram and run the blank sample. Bring the entire population of cells on scale by adjusting the gain. For fluorescence, locate the FL2 population completely on scale. Run the viability dye sample. For single parameter fluorescence viability determinations, place a region around the FL2 non-fluorescent population to report the percent viability. For dual parameter viability determinations using volume and fluorescence, place regions around distinct population distributions to report the percent viability.
**Instrument Configuration**

- 488 nm Laser
- 560 Short Pass Dichroic
- 525/40 Band Pass (FL1)
- 570 Long Pass (FL2)

**Selected References**

1. Princeton University, Department of Molecular Biology. *Flow Cytometric Protocols for Cell Viability Assays.*


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**Figure 1.** Cell Lab Quanta™ Optical Configuration with 488 nm Diode Laser.
Figure 2. PB6 Tissue Culture Cells (Unstained Control). The non-fluorescence signal is set to the first decade and used as the negative baseline.

Figure 3. PB6 Tissue Culture Cells stained with Propidium Iodide. Single parameter fluorescence used to determine viability (the negative baseline was defined with the control sample).

Figure 4. PB6 Tissue Culture Cells stained with 7AAD. Dual Parameter volume and fluorescence used to determine viability (the negative baseline was defined with the control sample).

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