CytoFLEX Flow Cytometer

Technical Note

VIABILITY ASSESSMENT OF CELL CULTURES USING THE CYTOFLEX

The CytoFLEX* benchtop flow cytometer offers a simple and accurate method for determining the percentage of live and dead cells in growing cell cultures when a viability dye is added to the sample. An aliquot of the sample can be taken and run directly on the CytoFLEX to set up the negative gates for the viability dye and to also to determine cell concentration. Once cell concentration is known, the appropriate amount of dye can be added to the sample and run on the instrument in order to determine the viability of the population.

Materials & Methods for viability using 7AAD on the CytoFLEX

- Tissue culture cells grown in appropriate media
- Viability dye

Cell Preparation with 7AAD (stock solution 1mg/mL)

1. If the concentration of the cells are lower than 1 x 10e4 cells/mL, the cells should be concentrated and re-suspended in PBS to a final concentration of ~1e6 cells/mL
2. If the concentration of the cells are at ~ 1e6 cells/mL, the 7AAD can be directly added to the cells in the tissue culture media
3. Use 20 µL 7AAD for every 1mL of cells. NOTE: The concentration of 7AAD may need to be adjusted depending on the cell culture type.
4. Mix and read within 5-10 minutes on the CytoFLEX cytometer

CytoFLEX Instrument Set–Up

INSTRUCTIONS:

1. Create a new experiment from the menu for the viability test samples.
2. Create two dot plots, one FSC vs SSC and the second 7AAD (PC5.5 channel) vs SSC
3. Set the 7AAD axis to log mode
4. Run the sample at the Low flow rate setting—10ul/minute
5. Set the threshold on the FSC channel.
6. In the Statistics plot add the events/µL parameter
7. Adjust the gain settings for all three parameters with the unstained sample
8. Draw a gate on the entire FSC vs SSC population excluding the debris in the lower left hand corner of the plot
9. Set this gate on the 7AAD vs SSC plot
10. Draw a gate on the negative 7AAD population
11. Run the test sample with 7AAD added
12. Collect 40,000 events total
13. Calculate the viability based on the gating hierarchy for the population in the 7AAD or negative gate
    a. You can also verify the cell concentration by looking at the events per µL and multiplying it by the total volume in the tube.

Figure 1.

CHO cells were prepared as described and run on the CytoFLEX.
Unstained sample on the left for reference; 7AAD stained on the right.

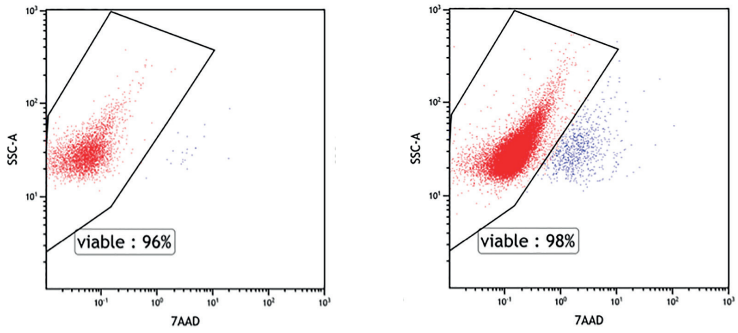


Figure 2.

HeLa cells were prepared as described and run on the CytoFLEX.
Unstained sample on the left for reference; 7AAD stained on the right.

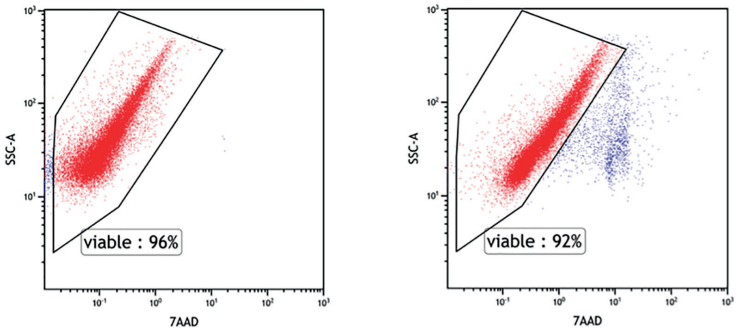


Figure 3.

293T cells were prepared as described and run on the CytoFLEX.
Unstained sample on the left for reference; 7AAD stained on the right.

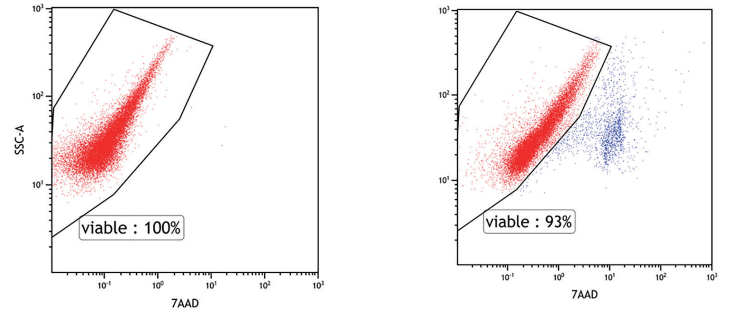
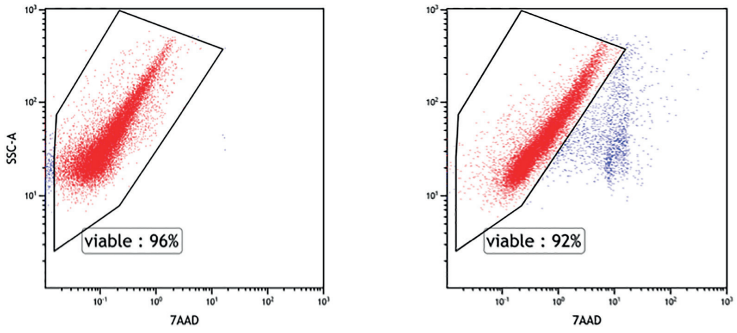


Figure 4.

721 cells were prepared as described and run on the CytoFLEX.
Unstained sample on the left for reference; 7AAD stained on the right.



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