Flow cytometry analysis and sorting of Xdrop DE50 droplets containing cells

This document should be treated as a guide and may require adaptation depending on flow cytometers, fluorochromes, and cell types. This protocol is designed for cells encapsulated in Xdrop DE50 droplets.

Requirements

- 100 µm nozzle tip or larger for flow cytometry analysis
- 130 µm nozzle tip or 130 µm nozzle sorting chip for sorting

Settings and considerations

Droplet density: Due to its high density, DE50 droplets will quickly sediment to the bottom of the sample tube. Adjust the sample probe to hover just above the bottom of the sample tube during analysis or sorting. Run with a high sample pressure to draw the droplets into the channels of the flow cytometer.

Note that it can take 5 to 10 minutes for the DE50 droplets to reach the point of interrogation and appear on the plot.

Droplet diameter: Despite the larger outer diameter of DE50 droplets (70 µm) compared to what is usually recommended on 100–130 µm nozzles, flow cytometry remains feasible due to droplet flexibility and low likelihood of aggregation. Use a high sample pressure or fast flow rate to increase the width of the inner sample stream.

Assay chemistry: The oil shell of the DE50 droplets is transparent and will permit detection of light from inside the droplet. Ensure assay chemistry within the DE50 droplets contains fluorophores compatible with the detection spectrum of your flow cytometer.

Threshold trigger channel: During double emulsion generation, oil only droplets may be produced. Use forward scatter (FSC) as the threshold channel to exclude unwanted events such as oil droplets and debris.

Doublet discrimination: Doublet discrimination can be performed but is not required as DE50 droplets do not aggregate.

Sample reanalysis: As DE50 droplets may have broken or bleached during sorting, reanalysis of the sorted population is not recommended as a method for assessing purity. A small volume of DE50 droplets can be sorted directly onto a microscope slide, for microscopic inspection and purity assessment.

Preparing DE50 droplets for flow cytometry

1. After droplet production or incubation, collect the DE50 droplets in a 2 ml DNA low bind centrifuge tube as described in the Xdrop Manual[1].

2. Remove the outer buffer and dilute DE50 droplets at a 1:5 ratio in the appropriate diluent (media + stabilizing solution). Ensure the diluent and droplets are at the same temperature.

Choose the diluent according to these guidelines:

Media + 25% DE stabilizing solution for DNA 4x (REDIVSTABSOL0900): This solution provides optimal stabilization for the droplets but contains Tween-80, which could be toxic for cells in case of exposure longer than a few hours.

Media + 33% DE stabilizing solution for cells 3x (REDIVSTABSOL1500): This solution provides stabilization for the droplets and is not toxic for cells. This solution is recommended if the sorted cells are going to be expanded or for sensitive downstream analyses, such as gene expression analysis. 10% Optiprep™ may be added to increase the density of the of the diluent decreasing droplet sedimentation rate.

3. Gently swirl or flick the tube before loading the DE50 droplets into the flow cytometer.
   Note that manual resuspension may be required during a run if the event rate drops significantly.

4. *If sorting*, add 200 µl of the droplet diluent to the bottom of a 2 ml collection tube and place it in the appropriate holder.

5. Load the sample tube and start analysis.

Droplet flow cytometry analysis and sorting

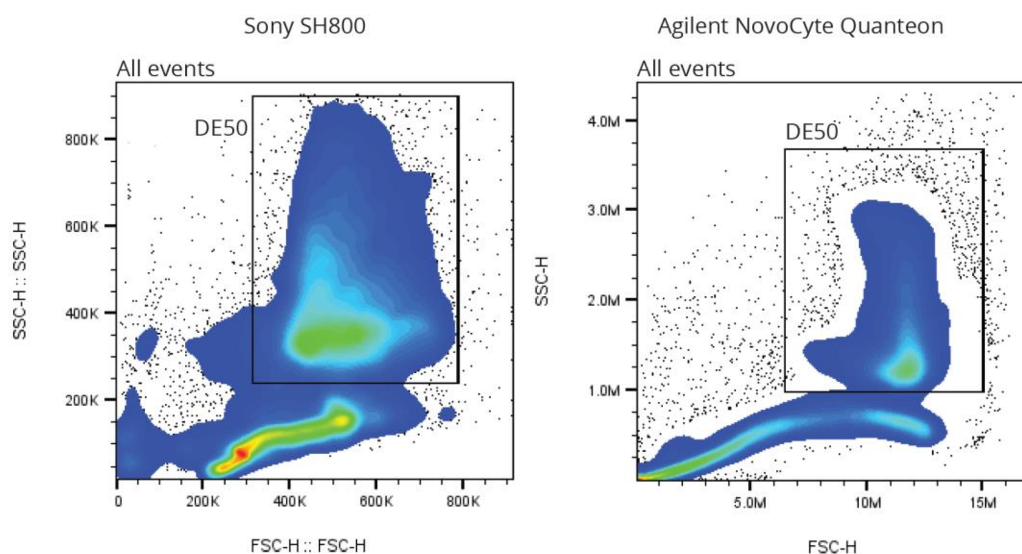

Figure 1. Detection of DE50 droplets containing cells using forward scatter (FSC) and side scatter (SSC) on two different flow cytometers, Sony® SH800 Cell Sorter (left) and Agilent® NovoCyte Quanteon (right). DE50 droplets can be identified as a distinct population separate from pure oil droplets and debris when the entire droplet production is analyzed. The plots may differ depending on the instrument and on the encapsulated material.

1. Run with a high sample pressure/flow rate waiting up to 10 minutes for the DE50 droplets to appear on the plot.

2. Use FSC as the threshold trigger channel to exclude background noise.

3. Display at least 100,000 events on the live plot to ensure that rare events are trackable.

4. Identify DE50 droplets using the FCS-height vs. SSC-height plot as shown on Figure 1. Note that plots may differ depending on the flow cytometer used.

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5. Set up new plots from the DE50 gate with fluorescence as shown in Figure 2. Use DE50 droplets with unstained sample in similar media as a negative control to set the sorting gates (Figure 2).

6. Confirm sort settings and check that the side stream is centered on the collection tube.

7. Start sorting the population(s) of choice and acquire data during the sort for your records.

8. After sorting, briefly spin the collection tube and store at room temperature. To keep the droplets intact, do not store at 4°C.

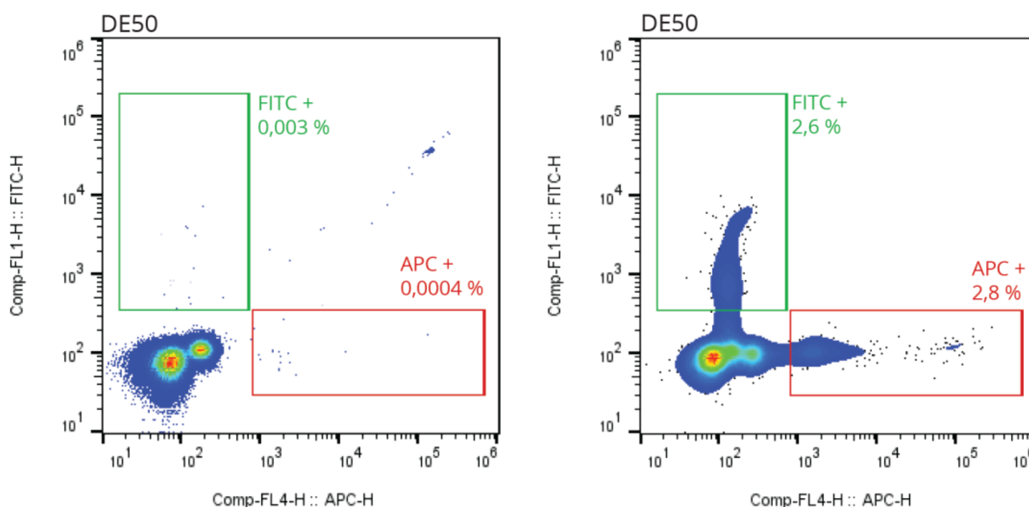

Figure 2. Flow cytometry analysis of DE50 droplets containing cells stained either with APC (FL4, red) or FITC (FL1, green) on Sony® SH800S Cell Sorter. Left: APC vs. FITC analysis of DE50 droplets containing no fluorescent cells (control). Right: Analysis of DE50 droplets containing cells labelled with APC (red box) or FITC (green box).

References

1. Xdrop Manual
https://samplix.com/support#manuals

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