Samplix

Flow cytometry analysis and sorting for DE50 droplets containing cells

This document should be treated as a guide. Adaptation may be needed depending on the fluorochromes and cell type. This protocol is for Xdrop double-emulsion droplets with an inner diameter of 50 µm and an outer diameter of 70 µm (DE50 droplets) that contain cells.

Requirements for flow cytometer

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

Position the sample probe just above the bottom of the sample tube. Set the lasers, filters, and compensation strategy to suit your chosen fluorochromes.

Considerations for flow cytometry with DE50 droplets

Droplet weight: DE50 droplets are stable and relatively heavy. Start 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 density: Due to the density of the DE50 droplets, they will quickly sediment at the bottom of the sample tube and will not remain in suspension during sorting. This is generally not a problem provided the sample probe is positioned just above the bottom of the sample tube.

Droplet diameter: The outer diameter of the DE50 droplets (70 µm) is larger than usually recommended for flow cytometry on 100–130 µm nozzles. However, since the DE50 droplets are flexible and do not aggregate, flow cytometry is still possible.

Required assay chemistry: The assay chemistry within the DE50 droplets should emit fluorescent light compatible with your flow cytometer's detection spectrum. The oil shell of the DE50 droplets is transparent.

Threshold trigger channel and doublet discrimination: Use forward scatter (FSC) as the threshold channel to exclude contaminating events such as particles in the buffer. Doublet discrimination can be performed but is not required as DE50 droplets do not stick together.

Reanalysis: DE50 droplets may be deformed, broken, or bleached during sorting. Therefore, a flow cytometry reanalysis of the sorted DE50 droplet population is not recommended as a method for assessing purity.

Preparing DE50 droplets for flow cytometry analysis and sorting

1. Dilute the DE50 droplets 1:5 in dPBS with 2% FBS (or 0.5% BSA) in a 5 ml (12 x 75 mm) round bottom tube.

2. 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.

3. Add 200 µl dPBS with 2% FBS into the bottom of a 1.5 ml collection tube and place the collection tube in the appropriate holder in the collection area of the instrument.

4. Load the sample tube containing the DE50 droplets in buffer onto the flow cytometer and start analysis.

Flow cytometry analysis and sorting of DE50 droplets

1. As explained above, start with a high sample pressure to draw the droplets into the channels of the flow cytometer and wait up to 10 minutes to see the DE50 droplets in the plot.

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

3. Use FSC as the threshold trigger channel to exclude background noise, e.g., particles from the buffer.

4. Discriminate DE50 droplets with stained cells inside from unstained droplets by creating a plot showing fluorescence at a relevant wavelength vs. fluorescence at another e.g., FL1 vs. FL 4 (Fig. 1).

5. Use DE50 droplets with an unstained sample in similar media as a control to set the sorting gates (Fig. 1).

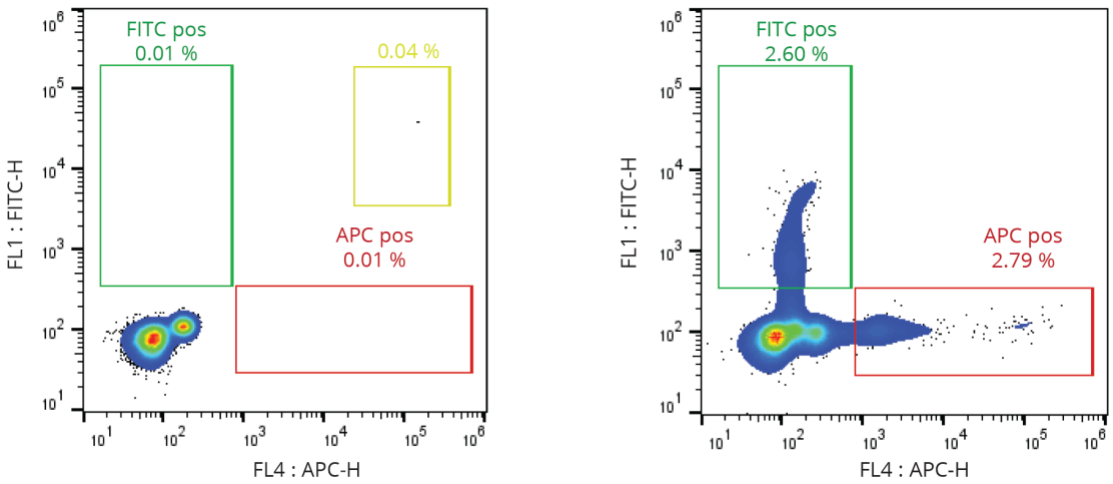

Figure 1. Example of flow cytometry sorting with two colors: APC (FL4, red) and FITC (FL1, green). Left: APC vs. FITC analysis of DE50 droplets containing no fluorescent stain (control). Right: Analysis of DE50 droplets containing cells labelled with APC (red box) or FITC (green box). Sorted on a Sony® SH800S Cell Sorter.

6. Confirm that the sort settings for your cell sorter are correct. Check that the side stream is centered on the collection tube.

7. Start sorting the population(s) of choice into a collection tube containing 200 µl dPBS with 2% FBS. Acquire data during the sort for your records.

8. After sorting, keep the tube with the sorted droplets at room temperature.

Breaking sorted DE50 droplets containing mammalian cells

Note: This protocol assumes ~100 µl of droplets in the collection tube at the start of the protocol. Do not use larger amounts. If you are using smaller amounts, reduce the amounts of all other reagents in proportion (e.g., if using 50 µl of droplets, then add 250 µl of dPBS, 50 µl of Droplet break solution, etc.).

This protocol should be treated as a guide. Adaptation for various cell types and growth media may be needed to ensure the survival of the cells after their release from the droplets. We have performed the described protocol both at room temperature and at 4°C by precooling the dPBS and keeping the droplets and cells on ice.

1. Wash the droplets twice with the dPBS, following this process both times:
    a. Add 1 ml of dPBS to the droplets and invert the tube.
    b. Let the droplets sediment, which normally happens within 30 seconds.
    c. Remove the dPBS, taking care not to disturb the pellet of droplets.
2. Add 500 µl of dPBS to the droplets.
3. From this step on, process only one sample at a time. Add 100 µl of Droplet break solution ● to the droplets, flick the tube 10 to 20 times, wait 1 min, and spin briefly (3–5 sec) at 400 g.
4. Transfer the upper phase, which contains the cells, to a 2 ml Eppendorf® tube.
5. Add 200 µl dPBS to the leftover DE50 droplets in the collection tube, then add 100 µl Droplet break solution ●, flick the tube 10 to 20 times, wait 1 min, and spin briefly (3–5 sec) at 400 g.
6. Transfer the upper phase, which contains the cells, to the same 2 ml Eppendorf tube as above.

7. Repeat steps 5 and 6 if there are any droplets left in the collection tube. They are easy to see as a layer between the upper and lower phases.
8. The released cells are now all contained in the 2 ml Eppendorf tube. Pellet the cells at 400 g for 5 min.
9. Using a pipette, carefully remove the supernatant. Note that the pellet is loose because of the oil.
10. Add 1 ml dPBS and resuspend the pellet.
11. Pellet the cells at 400 g for 5 min, remove the supernatant, and resuspend the cells in 250 µl growth medium.