Breaking DE50 droplets containing cells

Materials

- Collection tube and 2 ml Eppendorf® tube
- DE50 droplets containing cells
- Dulbecco's phosphate-buffered saline (dPBS)
- Droplet break solution ●
- Growth medium (prefer the same one used during the droplet production process)

Method

Note: This protocol assumes ~100 µl of droplets in the collection tube at the start of the protocol. Scale down for smaller amounts. Do not use larger amounts.

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 PBS 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.

Please refer to the Xdrop Manual or Xdrop Sort Manual for more information.