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Biomedical Engineering

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Protocol Droplet Separation

This is a droplet separation protocol for the extraction of polyacrylamide droplets. Two methods of separation – centrifugation and filtration – are used. The protocol has been composed with the use of scientific resources and is improved by trial and error during the experiments.

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Droplet Separation

1 Centrifugation I

- Take the eppendorf tube where the droplets are stored.
- Add with 20% v/v of PFO (Perfluoroctane) in the HFE-7500.
- Gently mix the PFO with the oil phase by tapping the eppendorf cup.
- Leave the cup on the work bench for 1-2 minutes. Once the droplets are broken, the two phases become clear and the beads are separated from the oil phase.
- Spin the tube at 60 × g for 30 seconds to fully separate the two phases.
- Add distilled water on a 1:1 ratio.
- Since the droplets are located on the separation layer of the oil and continuous phase, pipet the oil phase away until only the water (with the droplets) is present in the eppendorf tube.
- Put 5 µL of the solution (pipet on the bottom of the eppendorf tube) on a glass slab to analyze further under the microscope. Cover the solution sample with a coverslip.

2 Separation

- Remove the mineral oil from the top of the collected particles.
- Centrifuge at 3000 × g for 30 seconds.
- Remove bottom oil phase.
- Add 500 µL of neat HFE-7500, vortex and centrifuge.
- Remove bottom oil phase.
- Add 500 µL of HFE-7500 with 20% v/v perfluorooctane, vortex and centrifuge.
- Remove bottom oil phase.
- Add 1 mL 1 w/t% span-80 in hexanes, vortex and centrifuge.
- Remove upper hexane phase.
- Add 1 mL 0.1 w/t% triton X-100 in water.
- Pipette up and down, vortex or sonicate to disperse particles, centrifuge.
- Remove upper continuous phase including any oil/water emulsion floating on top of the liquid.
- Add 1 mL 0.1 w/t% triton X-100 in water.
- Pipette up and down, vortex or sonicate to disperse particles, centrifuge.
- Disperse in plain water.

3 References

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