**Video Article**

# Title Cell Encapsulation by Droplets

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## Abstract

## Protocol

## NIH3T3 cells preparation:

**A. Cells for Ejection**

1. Trypsinize cells, then dilute 1:1 with cell media, and transfer from a T75 flask to a 15 mL Falcon tube
2. Spin down cells into a pellet by centrifuging, aspirate supernatant and wash cells with DPBS
3. Spin down cells into a pellet again, and aspirate supernatant
4. Resuspend cells in media
5. Determine cell density with hemocytometer (~200 X $10^4$ cells/mL per T75 flask)
6. Centrifuge cell solution, aspirate supernatant, and resuspend in appropriate amount of media for varying cell concentrations

**B. Cell ejection**

1. Vortex cells before using for ejection
2. Transfer 200 µL of cell solution into syringe
3. Set appropriate mode on pulse generator
    1. For ejecting single droplets and multiple droplets (bursts), set pulse generator to "E. BUR" mode
    2. For continuous droplet ejection, set pulse generator to "NORM" mode
4. Change signal settings
    1. Set high level and low level output voltage: HIL to 5 V and LOL to 0 V and make sure the"LIM"LED is on
    2. Set signal as a square pulse
    3. Change the amount of time the solenoid valve is open for droplet ejection by changing the value for "WID" or changing duty cycle ("DUTY")
    4. Change the frequency of ejection by changing the value for "PER"
    5. Change the number of droplets ejected in a burst by changing the value for "BUR"
5. Eject cell solution onto prepared substrate for imaging with microscope

**C. Staining**

1. Make up dye solution with 0.5 µL calcein-AM and 2 µL ethidium homodimer per mL of DPBS
2. Immerse prepared substrate in dye solution
3. Allow sample to incubate for 10 minutes at 37°C before imaging

## Experiment Validation

1. On a Nikon Eclipse TE-2000 U Fluorescent Microscope
    1. Spot advanced software (Diagnostics, Inc.)
    2. Live/Dead Assay

## References