

HPTS-working protocol

Monday, October 29, 2018
1630

To a 50-mL beaker add:

5 mg Cholesterol (dissolve into chloroform solution of POPC) (100 uL)

200 uL 25 mg/mL POPC (5 mg)---vortex

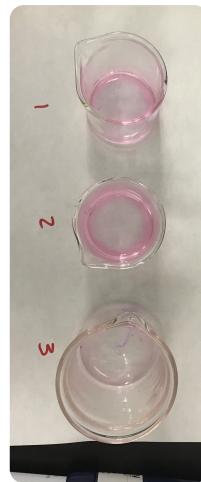
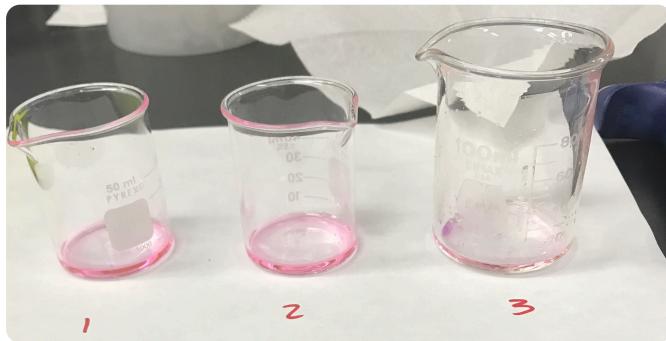
10 uL 1 mg/mL Rho-DPPE (~0.25 mol%)----vortex

Add 1 ml mineral oil----vortex

Heat at 80C for 15 min (vortex)

Cool oil for 5 min on the bench (paper-covered bench)

Images of lipid and oil mixture after cooling



3 X 650 →

Incubate the following in a microfuge-tube rack for 10 min at 4C:

150 uL of lipid/oil on top of 225 uL outer solution

2 tubes of 225 uL of outer solution

1 tube 400 uL of outer solution (Based how much you resuspend in)

20 uL inner solution (1 uL HTPS (40 uM) and 19 uL inner solution)

500 uL of lipid/oil

Cool centrifuge to 4C



To 500 uL of 4C lipid/oil mixture
add 20 uL inner solution (1.5 uL HTPS and 28.5 uL inner solution)
Pulse using a ~~mini sonicator~~. 5 very brief pulses, just squeeze and let go
vortex, vortex for 2 minutes

dispersion appears complete

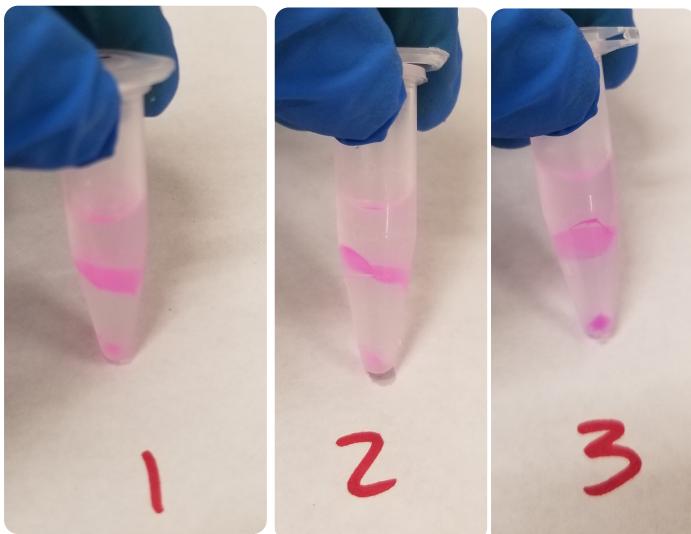
No droplet visible on the bottom

Keep on ice while not handling



Layer over the 225 uL cold outer solution (200 mM glucose, 100 mM HEPES pH :sunglasses: that was preincubated with 150 uL cold lipid/oil

Centrifuge immediately at 14000 g for 10 min at 4C

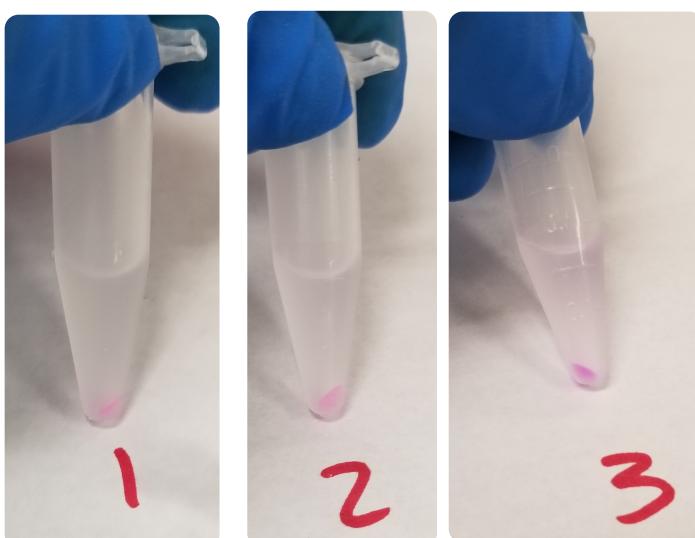


Carefully remove the oil layer with 1-mL tip and finally as much as possible with a gel loading tip

Transfer the pellet into another 225 mL outer solution using a 1 mL pipette tip

~~Mix by pipetting up and down using 1 mL pipette tip~~ (missed this part)

Centrifuge at 9000 g for 5 min at 4C

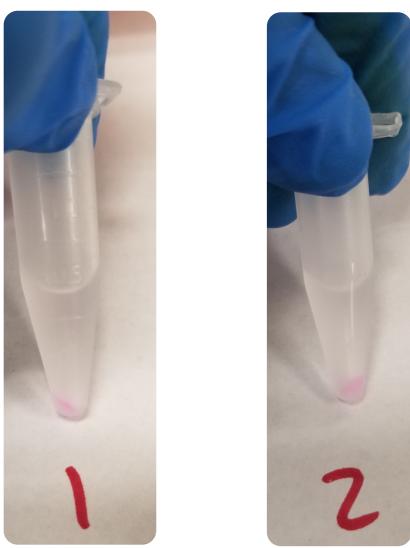


Carefully remove the oil layer with 1-mL tip and finally as much as possible with a gel loading tip

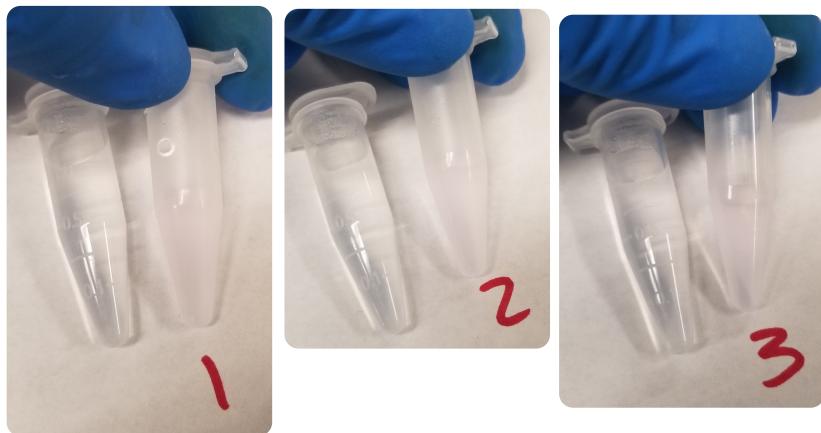
Transfer the pellet into another 225 uL outer solution using a 1 mL pipette tip

~~Mix by pipetting up and down using a 1 mL pipette tip~~

Centrifuge at 9000 g for 5 min at 4C



Transfer the pellet into 400 uL outer solution using a 1 mL pipette tip
Mix by pipetting up and down using a clean 1 mL pipette tip



Right microcentrifuge tube is H₂O for clarity comparison.

Place 9 uL on a ~~poly-L-lysine-coated~~ slide topped with a 0.12 mm spacer

Do Microscopy as soon as possible (within minutes)

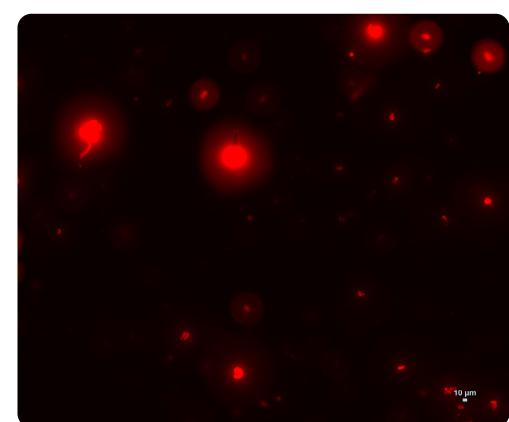
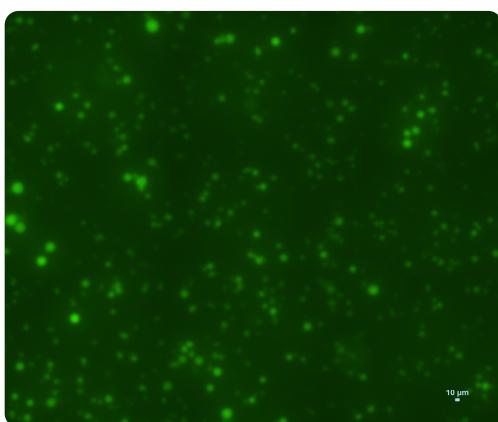
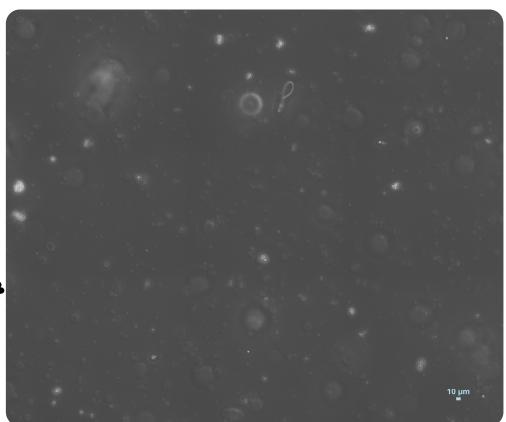
Channels

BF

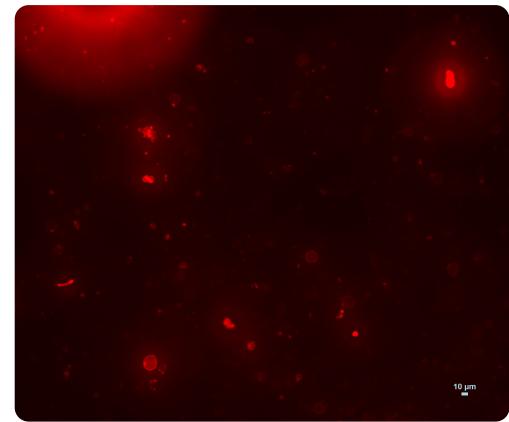
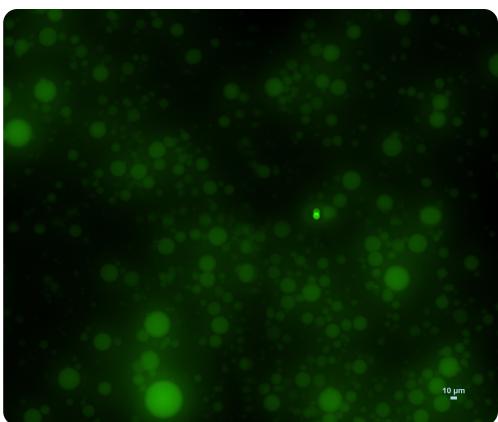
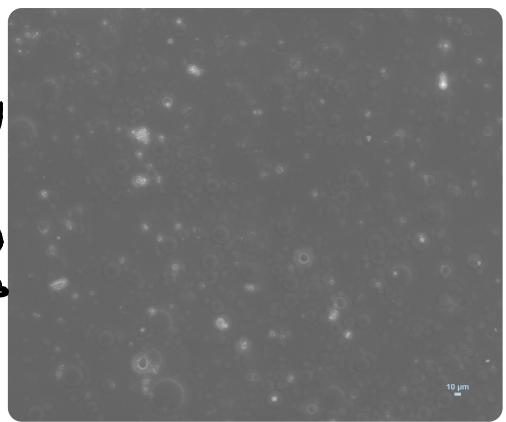
GFP

RFP

Sample # 1



Sample # 2



Sample # 3

