RNA Isolation in Levitated 3D Cultures

Before you start

- Clean working area, tube racks, and pipettors with a RNAse decontamination reagent
- TRIzol should only be used in the fume hood and with proper safety gear, including gloves, lab coat, and goggles. Have a dedicated TRIzol waste receptacle that can be sealed to collect all waste that touches TRIzol.

Supplies

- Nuclease-free barrier tips and 1.5 mL centrifuge tubes
- Bucket of ice
- 0.5 mL screw cap tubes

Reagents

- TE buffer
- TRIzol
- Chloroform
- Isopropanol (IPA)
- 75% ethanol (make fresh from designated RNA-only 200 proof ethanol diluted in nuclease-free H_2O
- Cold sterile PBS
- Cold nuclease-free H_2O

Protocol

1. In a sterile culture hood, open well plate with 3D cultures, and remove the magnetic drive and lid insert. Place the magnetic drive underneath the plate with the magnets facing upwards to attract and hold the 3D cultures at the bottom of the well. Wash the cultures 2X with cold PBS and aspirate as much liquid as possible without disrupting the culture. Transfer the plate to a fume hood.
2. Add TRIzol to each well in volumes according to the following table:

	TRIzol volume (µL)
24-well	500
96-well	250

   Remove the plate off the magnetic drive and use repeated and vigorous pipette action to break apart the 3D culture. After the 3D culture is thoroughly broken apart, transfer the solution to a centrifuge tube, pipette vigorously again several times and incubate inside the hood for 5 min.
3. Add 200 µL of chloroform per tube and mix vigorously with tube in hand in a windshield wiper motion for 30 sec. Incubate inside the hood for 3 min, then centrifuge the tubes at 4°C and 11,000 rpm (12,000 g) for 15 min.
4. After centrifugation, remove the colorless upper aqueous phase and transfer it to a new tube, which should include the RNA. One can also place the tube over

5. the magnetic drive to ensure that the remaining tissue stays at the bottom. Add 500 μL of IPA, and again mix vigorously by hand as described in Step 3 for 30 sec. Let the solution incubate for 10 min in the hood and centrifuge the tubes at 4°C and 11,000 rpm (12,000 g) for 10 min.
6. Carefully remove the supernatant from the tube without disturbing the white RNA pellet in the tube. Wash the pellet with 75% ethanol and vortexing. Centrifuge again at 4°C and 9,200 rpm (7,500 g) for 5 min.
7. Remove as much supernatant as possible from the tube without disturbing the pellet. Let the tube rest upside down to dry at room temperature.
8. Label 0.5 mL screw-cap tubes and cool them beforehand on ice. Suspend RNA pellets in 20 μL cold nuclease-free H_2O per mL TRIzol used. Once dissolved, keep the sample on ice.