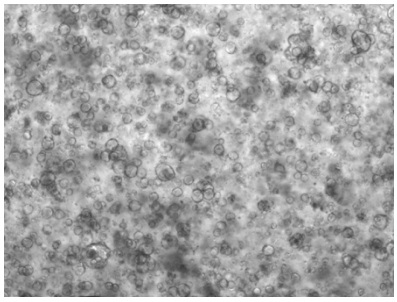
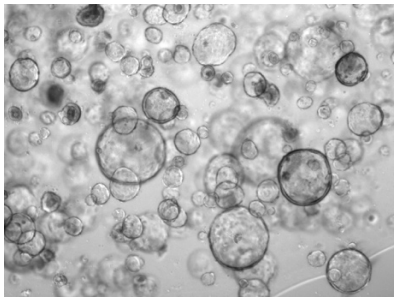
Reagents and equipment

- Cell Recovery Solution (CRS) - 1X, Corning #L004419-CPB40253
- Dulbecco's PBS, no Ca+2 or Mg+2 (PBS), Gibco #14190-144
- 15 ml conical tubes, Falcon #F2097
- 10ml plastic pipettes, Falcon #F7551
- Pipet aid
- 200 µL pipette tips with filter, Corning #4138
- 1000 µL pipette tips with filter, Corning #4140
- Refrigerated 15 mL tubes centrifuge, Thermo Fisher Scientific #IEC CL31R Multispeed Centrifuge
- Refrigerated 1.5 mL tubes centrifuge, Thermo Fisher Scientific #MICROCL 21R
- 1,5 ml tubes, Eppendorf #L000265-286730000
- Cell Lysis Buffer (10X), CST #BK9803S
- cOmplete, Mini Protease Inhibitor Cocktail, Roche #4693124001
- PhosSTOP, Roche #4906845001
- Ice bucket with ice
- Rotator

Procedure to prepare organoid pellet for protein extraction

In order to obtain ~400 µg of proteins, it is suggested to collect at least 8 domes (estimated cell number 1.5 X 10^6 - 3 X 10^6, depending on the culture). Perform the entire procedure on ice. Below, microphotographs showing the targeted density for pelleting organoids.

1. Label a 15 mL tube and a 1.5 mL tube with the ID of the culture and put them on ice. Pre-fill the 15 mL conical tube with 2 mL of ice-cold CRS.
2. Prepare 10mL of PBS 1X containing half tablet of PhosSTOP (PBS-P) and place it on ice. The total amount of PBS-P will depend on the number of collections. In general, 10 mL of PBS-P is used to prepare pellet from one well of a 6-well plate.
3. For an individual well of a 6-well plate (8 domes, 50µL each), carefully aspirate the culture medium with a vacuum aspirator and then add 2 mL of ice-cold CRS to the well.
4. Pipet up and down the solution with a p1000 to dissolve Matrigel and transfer the entire volume to the pre-labeled 15 mL tube
5. Wash the plate with 1 mL of ice-cold PBS-P to collect any leftover.
6. Add enough PBS-P (around 6mL) to bring the solution to 10 mL.
7. Incubate on ice for 30 minutes, pipetting up and down and inverting the tube after 10 minutes of incubation
8. At the end of the incubation, centrifuge for 5 minutes at 300 at 4°C
9. Carefully aspirate the supernatant and add 1 mL of PBS-P
10. Resuspend the pellet and transfer it to the 1.5 mL tube
11. Centrifuge the tube for 10 minutes at 10.000 g, 4°C
12. Aspirate the supernatant without disturbing the pellet, then put the pellet on ice; at this point it is possible to directly proceed with cell lysis, otherwise the pellet should be stored at -80°C