Subculture and expansion of human organoids

Read this protocol in its entirety before proceeding

Passaging organoids for expansion

1. Place a 6-well culture plate in a 37°C incubator to warm for at least 1 hour.

2. Prepare a 10 mM working solution of ROCK Inhibitor Y-27632 (ROCKi; ATCC® ACS-3030™) by adding 3 mL sterile water to a 10 mg vial of ROCKi.

3. Warm the appropriate growth medium at room temperature.

4. Thaw the appropriate extracellular matrix on ice or at 2-8°C. Large volumes (multiple mL) of ECM (extracellular matrix) may take 12-24 hours to thaw. Avoid multiple freeze/thaw cycles of the ECM.

5. Aspirate media from culture vessel containing organoids.

6. Scrape domes from vessel surface using a P1000 pipette tip or cell scraper.

7. Transfer detached domes to a conical tube.

8. With a P1000 pipettor, pipette up and down 20-30X to break up the domes.

9. Fill the tube to max volume with cold growth medium.

10. Spin the tube at 300 x g for 5 minutes at 4°C.

11. Aspirate the supernatant.

12. Re-suspend the pellet in TrypLE (Thermo Fisher Scientific catalog #12604013) and place in a 37°C water bath for 5-15 minutes or until 80% of the organoids have been reduced to single cells. Monitor disruption with an inverted microscope.

    Optional: Take a sample to count.

13. Fill the tube to max volume with cold growth medium.

14. Spin the tube at 300 x g for 5 minutes at 4°C.

15. Re-suspend the pellet in an appropriate volume of ECM by pipetting up and down 20-30 times.

16. Using a P200 pipette, aspirate 100 µL the ECM/cell suspension and dispense as small droplets in the surface of a single well in the 6-well plate. You should end up with approximately 8-12 droplets in the well.

17. Repeat for all the remaining ECM/cell suspension, dispensing 100 µL per well as small droplets.

18. After all of the suspension has been seeded, place the lid on the plate and invert (turn upside down). Place the plate, still inverted, in the cell culture incubator for 15-20 minutes to solidify the ECM.

19. While the ECM is solidifying, supplement the remaining 10 mL of pre-warmed complete growth medium with ROCKi to a final concentration of 10 µM. For example, add 10 µL of a 10 mM solution of ROCKi to 10 mL of growth medium.

20. After the ECM has solidified, return the plate to a BSC and flip right side up.

21. Add 2 mL per well of pre-warmed complete culture media containing 10 µM ROCKi. Dispense the media along the wall of the well, not directly on the domes.

22. Return the 6-well plate to the cell culture incubator.

23. Every 2-3 days perform a medium change with pre-warmed complete growth medium.

For a more detailed protocol on culture of organoids, please see the following publication:

Clinton J, McWilliams-Koeppen P. Initiation, Expansion, and Cryopreservation of Human Primary Tissue-Derived Normal and Diseased Organoids in Embedded Three-Dimensional Culture. *Curr Protoc Cell Biol* (2018): e66. PubMed: 30265443 https://currentprotocols.onlinelibrary.wiley.com/doi/10.1002/cpcb.66

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