Cryopreservation of human organoids

Read this protocol in its entirety before proceeding

Cryopreserving organoids

1. Aspirate medium from vessel containing organoids to be cryopreserved.
2. Scrape ECM domes containing organoids from vessel surface using a P1000 pipette tip or cell scraper.
3. Transfer detached domes to a conical tube.
4. With a P1000 pipettor, pipette up and down 20-30X to break up the domes.
5. Fill the tube to maximum volume with cold growth medium.
6. Spin the tube at 300 x g for 5 minutes at 4°C.
7. Carefully aspirate the supernatant and discard.
8. Re-suspend the pellet in cryopreservation media such as Stem Cell Freezing Media (ATCC® ACS-3020™).

   We recommend cryopreserving the contents of one the equivalent of a single well of a 6-well plate (100 µL of ECM containing organoids) in 1.0 mL of cryopreservation medium.
9. Mix well by pipetting.
10. Transfer 1.0 mL of the suspension per cryovial.
11. Freeze the vials at -80°C at a rate of approximately 1°C/min such as with a CoolCell® LX Alcohol-Free Cryopreservation Container (ATCC® ACS-6000™).
12. After 24 hours, transfer the vials to LN2 vapor phase storage.

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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