

Cryopreservation of Organoids

1. **Purpose:** This protocol describes freezing and thawing of organoids cryo-vial. For optimal results, cryopreservation should be performed when organoids are mature.
2. **Procedure:**
 - 2.1 Prepare all media and reagents required for this protocol. Place PBS without magnesium or calcium, DMEM/F-12 with 15 mM HEPES, and CELLBANKER® cryopreservation media (Cat. #11890) to cool on ice. Retrieve the plate containing the organoids to freeze.
 - 2.2 Count the number of mature organoids found in each well and combine the contents of multiple wells as needed to achieve 200 organoids in each cryo-vial.
 - 2.3 Remove the organoid growth medium from each well and replace it with 1mL of cold (2-8°C) PBS.
 - 2.4 Break up the BME2 extracellular matrix by pipetting up and down ten to twenty times with a PBS-wetted 1000µL pipette tip. Transfer suspensions containing 200 organoids, combining wells if necessary, to a single 15mL conical tube.
 - 2.5 Wash each well with 1mL of cold (2-8°C) PBS by pipetting up and down five times with a pre-wetted 1000µL pipette tip and transfer to the 15mL conical tube.
 - 2.6 Pellet the organoids by centrifuging at 290 x g for five minutes at 2 - 8°C. Remove and discard the supernatant, being careful not to disturb the organoid pellet.
 - 2.7 Wash the organoid pellet by re-suspending in 7 to 10 mL of cold DMEM/F-12 with 15 mM HEPES. Gently flick the tube, or gently pipette the contents, to help break down the pellet if needed. Centrifuge the suspension at 200 x g for five minutes at 2-8°C then carefully remove and discard the supernatant.
 - 2.8 Re-suspend the organoid pellet in cold (2-8°C) CELLBANKER® medium using 1mL of freezing medium per cryo-vial of 200 organoids.
 - 2.9 Using the same pipette tip, move the organoids suspended in the final storage cryo-vial. Place the cryo-vial directly into a rate controlled freezer.
 - 2.10 Transfer the freezing container to a -80°C freezer for 24 hours. Maintain frozen organoids at -80°C or transfer the cryo-vial to liquid nitrogen (-135°C) for long-term storage.

Notes:

- 1 *Work quickly to avoid prolonged exposure of non-frozen organoids to CELLBANKER®*
- 2 *We recommend use of rate-controlled freezer for cryopreservation of organoids or more sensitive tissue samples*

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