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 an 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