

Protocol

# Cryopreservation of gut biopsies using PentaHibe<sup>®</sup> Complete

Protocol generously provided by Zilbauer Group, Cambridge Stem Cell Lab, Cambridge University

## Materials and reagents

- HBSS (wo Ca<sup>2+</sup>, Mg<sup>2+</sup>), chilled 2-8°C
- PBS chilled 2-8°C
- Advanced DMEM/F12 supplemented with 0.5 U/ml P/S, 2 mM GlutaMAX, 10 mM HEPES, chilled
- PentaHibe<sup>®</sup> Complete, chilled 2-8°C
- Cryovials (1.8 mL), ice, 37°C water bath
- Freezing container providing 1°C/min cooling (e.g., CoolCell/Mr. Frosty or equivalent), 80°C freezer and LN<sub>2</sub> storage

## 1 Sample collection

1. Collect gut biopsies into a sterile tube containing cold HBSS
2. Keep samples cold (on ice/cold pack) until processing

## 2 Cryopreservation procedure

1. In a sterile hood, remove transport medium
2. Wash biopsies 3-5 times with cold PBS to remove debris
3. Place 2 biopsies (approximately 3 mm in size) into each cryovial (1.8 mL)
4. Add cold PentaHibe<sup>®</sup> Complete to fully submerge the biopsies (target volume: ~ 500 µl per vial).
5. Place cryovials in a freezing container to achieve ~ 1°C/min cooling
6. Transfer the container to a -80°C freezer for at least 24 hours
7. For long-term storage, transfer vials to LN<sub>2</sub> freezer

## 3 Thawing

1. Retrieve vials from storage and transport on dry ice to the culture hood as quickly as possible
2. Thaw in a 37°C water bath. Thawing should be done gently by swirling the sample
3. Remove when only a small ice crystal remains
4. Transfer biopsies and cryomedium into a tube containing cold Advanced DMEM/F12 (+P/S, GlutaMAX, HEPES)
5. Wash 3 times with cold PBS, to remove residual cryomedium
6. Proceed to downstream applications (e.g., EDTA incubation and dissociation for organoid derivation)

**We are here to answer your questions**

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