

## Organoid Cryopreservation Medium

### Introduction

Organoid Cryopreservation Medium optimizes the formulation of cryopreservation, can maintain the viability and function of organoids, and can be widely used in the cryopreservation of a variety of organoids.

### Components and Storage

Size	100 mL	Storage
Components		
Organoid Cryopreservation Medium	100 mL	4°C
Shipping: Blue ice	Shelf life: 1 year	

### Materials Required but Not Included

Products	Catalog number
Organoid Wash Buffer	K2846

### Protocol

Cryopreservation must be performed when the organoids are in optimal condition. Fast-growing organoids (up to 300-500  $\mu\text{m}$  in diameter) can be cryopreserved when they reach 150-200  $\mu\text{m}$  in diameter. Slow-growing organoids (up to 100-300  $\mu\text{m}$  in diameter) can be cryopreserved when they reach 100-150  $\mu\text{m}$  in diameter.

**During the cryopreservation, gently pipetting to ensure the integrity of the organoids.**

#### 1. Organoid harvest

- 1) Discard the medium and slowly add pre-chilled Organoid Wash Buffer to the wells (PBS containing 1% antibiotics can also be used).
- 2) Pipet Matrigel/Organoid/Medium mixture up and down to release organoid from the Matrigel.

**\*Note:** If finding that organoids are attached to the wall, scrape the organoid off with a pipette tip.

- 3) Transfer the mixture to a new 15 mL tube and add Organoid Wash Buffer to make up the volume to 12 mL.
- 4) Place the tubes in -20°C for 6 min or 4°C for 30 min.

**\*Note:** Brief periods of low temperature do not affect the activity of organoids, and Matrigel can also be removed by taking advantage of the properties of Matrigel dissolving at low temperatures.

5) Centrifuge at 300 g or 1500 rpm for 5 min, and discard the supernatant.

## 2. Organoid cryopreservation

- 1) Depending on the pellet volume, mix the pellet and Organoid Cryopreservation Medium at a ratio of 1:10.
- 2) After mixing, aliquot the organoids into cryopreservation vials, and perform cryopreservation by programmed cooling.

**\*Note:** Cryopreserved organoids can be stored at -80°C for one month, and for long-term storage, store the vials in a liquid nitrogen tank.

## ■ Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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