

# Regenix Intestine protocol



Regenix Intestine is composed of various basement membrane proteins separated from the intestinal tissues. Regenix Intestine can be utilized for two-dimensional (2D) and three-dimensional (3D) culture of intestinal epithelial cells. In particular, Regenix Intestine can provide an optimized environment for adult stem cells (AdSCs)-derived and pluripotent stem cells (PSCs)-derived intestinal organoids.

## Storage Instructions

- ⊙ Avoid storing Regenix Intestine on freezer doors or in frequently opened freezers.
- ⊙ After the initial thaw, aliquot Regenix Intestine into freezer-compatible tubes and store at -80°C. Minimize repeated freezing and thawing to maintain product quality.
- ⊙ Long-term storage after thawing is not recommended for optimal product integrity.
- ⊙ Frozen Regenix Intestine is stable for up to 2 years from the date of manufacture.

## Thawing Instructions

- ⊙ Regenix Intestine begins to gel at temperatures above 10°C.
- ⊙ Thaw for at least 4 hours at 2°C to 8°C, ensuring the vial is fully surrounded by ice.
- ⊙ During thawing, keep the ice bucket covered and place it in a cold room or at the back of a refrigerator for consistent temperature control.

## Instructions for 3D Culture of Intestinal Organoids

### ⊙ Preparation of Regenix Intestine

Thaw Regenix Intestine and gently mix by slow pipetting. If bubbles occur, centrifuge before use. Keep Regenix Intestine at 4–8°C during handling to prevent gelation above 10°C.

### ⊙ Organoid Resuspension

Before adding Regenix Intestine, carefully remove as much supernatant as possible from the prepared organoid pellet. Then, add Regenix Intestine and gently mix by slow pipetting to ensure uniform resuspension. Regenix Intestine is provided as a ready-to-use pre-gel solution. Dilution is not recommended, as it may prevent proper hydrogel formation.

### ⊙ Gelation

Dispense 30 µL of the mixture into each well of a 48-well plate and incubate at 37°C for 40 minutes to allow gel formation.

## ⊙ Medium Addition

Carefully add the appropriate volume of medium. If adding 300  $\mu$ L per well, dispense the medium slowly over 15 seconds to prevent disruption of the gel. The culture of organoids with Regenix Intestine may require the addition of 10  $\mu$ M Y-27632 during the first 1–2 days.

## **Subculture Instructions for Intestinal Organoids**

### ⊙ Preparation of Collagenase Solution

Prepare a 2 mg/mL solution of collagenase IV (600–800 U/mL) in basal medium. Other types of collagenase can be used, but their concentrations may need optimization.

### ⊙ Detachment of Regenix Intestine Droplets

Gently touch the side of the Regenix Intestine droplet with a 1000  $\mu$ L pipette tip to detach it from the bottom of the well plate.

### ⊙ Transfer of Regenix Intestine-Encapsulated Organoids

Cut off the tip of a 1000  $\mu$ L pipette tip with sterile scissors to create a 2.5–3 mm opening. Use this modified tip to transfer the Regenix Intestine-encapsulated organoids into a 15 mL conical tube. Using a 15 mL conical tube is recommended to prevent the organoid pellet from sticking to the microtube walls.

### ⊙ Collagenase Treatment

Carefully remove the supernatant and add enough collagenase IV solution to fully submerge the Regenix Intestine droplets (e.g., use 1 mL of collagenase IV solution per 6–8 Regenix Intestine droplets).

Incubate the 15 mL conical tube upright at 37°C for 1 hour. Do not exceed 1 hour, as prolonged incubation may damage the organoids.

### ⊙ Removal of Regenix Intestine and Washing

After 1 hour, a thin layer of Regenix Intestine will remain above the organoid pellet. Carefully aspirate this layer and wash the organoids twice with basal medium.

### ⊙ Re-Encapsulation

Re-encapsulate the organoids in Regenix and continue culturing under the same conditions as before.