

Regenix™ Intestine

PROTOCOL

Regenix™ Intestine is composed of various basement membrane proteins separated from 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 cell (AdSC)-derived and pluripotent stem cell (PSC)-derived intestinal organoids.

PROCEDURE

3D culture of intestine organoid using Regenix™ Intestine

01

Thaw Regenix™ Intestine for at least 4 hours by submerging the vial in an ice bucket and storing it in a 4°C refrigerator before use. Avoid multiple freeze/thaw cycles.

02

Mix Regenix™ Intestine by slowly pipetting; Be careful not to create air bubbles during this process.

03

Add Regenix™ Intestine to the cell pellet and resuspend evenly by slow pipetting.

Note It is recommended to remove as much of the supernatant as possible before adding the Regenix™ Intestine.

04

Dispense 30 µL of the mixture to each well of a 48-well plate, and then incubate at 37 °C for 40 mins.

Note For suspension cultures, dispense Regenix™ Intestine droplets on a sheet of Parafilm for easy detachment after gelation.

05

Add an appropriate volume of medium very slowly.

Note If you need to add 300 µL per well medium to each well, add the medium slowly and carefully over 15 seconds.

Note Culture of intestinal organoids with Regenix™ Intestine requires the addition of 10 µM Y-27632 in the first 1-2 days.

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PROCEDURE

Passage of intestinal organoids in Regenix™ Intestine

- 01** Prepare 2 mg/mL of collagenase IV (600 – 800 U/mL) in basal medium.
Note Different types of collagenase also work, but need to be optimized to the proper concentration.
- 02** Gently touch the side of Regenix™ Intestine droplet with a 1000 µL pipette tip to detach it from the bottom of the well plate.
- 03** Cut the tip off a 1000 µL pipette tip with sterile scissors to obtain an opening of 2.5–3 mm in diameter, and use it to transfer each Regenix™ Intestine encapsulating organoids to a 15 mL conical tube.
- 04** Gently aspirate the supernatant and add enough collagenase IV solution to fully submerge Regenix™ Intestine droplets. (e.g. Use 1 mL collagenase IV solution per 6–8 Regenix™ Intestine droplets.)
- 05** Incubate the 15 mL conical tube containing Regenix™ Intestine in an upright position in a 37 °C incubator for 1 hour.
Note Long incubation times of more than 1 hour can damage the organoids.
- 06** After 1 hour, a thin layer of Regenix™ Intestine above the organoid pellet can be seen. Carefully aspirate the layer of Regenix™ Intestine and wash the organoids twice with basal medium.
- 07** Re-encapsulate the organoids in Regenix™ Intestine and cultivate them in the same way as before.