

Regenix™ Lung

PROTOCOL

Regenix™ Lung is composed of various basement membrane proteins separated from lung tissues. Regenix™ Lung can be utilized for two-dimensional (2D) and three-dimensional (3D) culture of lung epithelial cells. In particular, Regenix™ Lung can provide an optimized environment for adult stem cell (AdSC)-derived and pluripotent stem cell (PSC)-derived lung organoids.

PROCEDURE

3D culture of lung organoid using Regenix™ Lung

- 01

Thaw Regenix™ Lung 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

Cut the tip off a 200 µL pipette tip with sterile scissors to obtain an opening diameter of 1.5-2 mm, and mix Regenix™ Lung by slowly pipetting; Be careful not to create air bubbles during this process.

Note Regenix™ Lung may have high viscosity, so there may be some difficulty in pipetting. If a lot of bubbles are generated after pipetting, centrifuge before use.
- 03

Add Regenix™ Lung 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™ Lung.
- 04

Dispense 30 µL of the mixture to each well of a 48-well plate, and then incubate at 37 °C for 40 mins.
- 05

Add the 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 kidney organoids with Regenix™ Lung requires the addition of 10µM Y-27632 in the first 1-2 days.

Regenix™ Lung

PROTOCOL

Regenix™ Lung is composed of various basement membrane proteins separated from lung tissues. Regenix™ Lung can be utilized for two-dimensional (2D) and three-dimensional (3D) culture of lung epithelial cells. In particular, Regenix™ Lung can provide an optimized environment for adult stem cell (AdSC)-derived and pluripotent stem cell (PSC)-derived lung organoids.

PROCEDURE

Passage of Lung organoids in Regenix™ Lung

- 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™ Lung 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™ Lung encapsulating organoids to a 15 mL conical tube.

Note It is recommended to use a 15 mL conical tube to avoid cell pellet sticking to the microtube wall.
- 04

Gently aspirate the supernatant and add enough collagenase IV solution to fully submerge Regenix™ Lung droplets. (e.g. Use 1 mL collagenase IV solution per 6–8 Regenix™ Lung droplets.
- 05

Incubate the 15 mL conical tube containing Regenix™ Lung upright 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™ Lung above the organoid pellet can be seen. Carefully aspirate the layer of Regenix™ Lung and wash the organoids twice with basal medium.
- 07

Re-encapsulate the organoids in Regenix™ Lung and cultivate them in the same way as before.