

# Regenix™ Kidney

## PROTOCOL

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

### PROCEDURE

#### 3D culture of kidney organoid using Regenix™ Kidney

01

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

**Note** Regenix™ Kidney 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™ Kidney 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™ Kidney.

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™ Kidney requires the addition of 10 µM Y-27632 in the first 1-2 days.

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### PROCEDURE

#### Passage of kidney organoids in Regenix™ Kidney

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

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

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

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

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