cellArtgen

Regenix™ Liver

PROTOCOL

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

PROCEDURE

3D culture of liver organoid using Regenix™ Liver

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

Note Regenix™ Liver may form a gel at temperature above 10°C.
The temperature must be lowered to between 4°C and 8°C throughout all handling processes to ensure depolymerization.

03 Add Regenix™ Liver 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™ Liver.

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 liver organoids with Regenix™ Liver requires the addition of 10µM Y-27632 in the first 1-2 days.

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PROCEDURE

Passage of liver organoids in Regenix™ Liver

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™ Liver 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™ Liver 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™ Liver droplets. (e.g. Use 1 mL collagenase IV solution per 6-8 Regenix™ Liver droplets.

05 Incubate the 15 mL conical tube containing Regenix™ Liver upright in a 37 °C incubator for 1 hour.

Note Incubation times longer than 1 hour can damage the organoids.

06 After 1 hour, a thin layer of Regenix™ Liver above the organoid pellet can be seen. Carefully aspirate the layer of Regenix™ Liver and wash the organoids twice with basal medium.

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

CAUTION

* Organoids cultured in Regenix™ Liver grow slower than in Matrigel, but there is no observable difference in final organoid size. It is recommended to set the analysis/ harvest timepoint 2-3 days later than when using Matrigel. (e.g. Organoids grown in Regenix™ Liver for 7 days have the same observable size as organoids grown in Matrigel for 5 days)