Regenix Liver protocol

Regenix Liver is composed of various basement membrane proteins separated from the 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 cells (AdSCs)-derived and pluripotent stem cells (PSCs)-derived liver organoids.

Regenix Liver ECM Product Selection Guide

- To ensure optimal performance in liver organoid culture, please refer to the following instructions when selecting the appropriate product:

Product Name	Catalog No.	Recommended Application
Regenix Liver ECM, Low Conc.	RLI201-5X1ML	Mouse-derived liver organoid culture
Regenix Liver ECM, Medium Conc.	RLI401-5X1ML	Human primary liver tissue-based organoid culture
Regenix Liver ECM, High Conc.	RLI601-5X1ML	Human PSC-derived hepatic cell culture

Storage Instructions

- Avoid storing Regenix Liver on freezer doors or in frequently opened freezers.
- After the initial thaw, aliquot Regenix Liver 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 Liver is stable for up to 2 years from the date of manufacture.

Thawing Instructions

- Regenix Liver 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.

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Instructions for 3D Culture of Liver Organoids

- Preparation of Regenix Liver

  Thaw Regenix Liver and gently mix by slow pipetting. For RLI401 and RLI601, due to their high viscosity, it is recommended to use a 200 µL pipette tip with the end cut to a 1.5–2 mm opening to reduce bubble formation. If bubbles occur, centrifuge before use. Keep Regenix Liver at 4–8°C during handling to prevent gelation above 10°C.

- Organoid Resuspension

  Before adding Regenix Liver, carefully remove as much supernatant as possible from the prepared organoid pellet. Then, add Regenix Liver and gently mix by slow pipetting to ensure uniform resuspension. Regenix Liver 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 µL per well, dispense the medium slowly over 15 seconds to prevent disruption of the gel. The culture of organoids with Regenix Liver may require the addition of 10 µM Y-27632 during the first 1–2 days.

Subculture Instructions for Liver 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 Liver Droplets

  Gently touch the side of the Regenix Liver droplet with a 1000 µL pipette tip to detach it from the bottom of the well plate.

- Transfer of Regenix Liver-Encapsulated Organoids

  Cut off the tip of a 1000 µL pipette tip with sterile scissors to create a 2.5–3 mm opening. Use this modified tip to transfer the Regenix Liver-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 Liver droplets (e.g., use 1 mL of collagenase IV solution per 6–8

Regenix Liver 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 Liver and Washing

  After 1 hour, a thin layer of Regenix Liver 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.

If you have any questions, please contact
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