Primary Tupaia Hepatocytes

（Cat# LV-PTH001/2）

(For research use only)

To ensure personnel safety and biosafety, appropriate personal protective equipment (PPE) must be worn at all times when handling this product and its waste, including protective masks, latex gloves, and goggles during cell resuscitation. Strictly follow the instructions provided in this manual. All experimental waste must be disposed of safely in accordance with national, provincial, and municipal regulations to maintain biosafety standards.

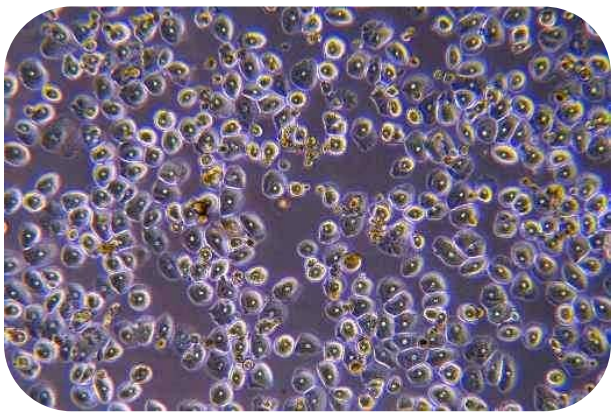
I Introduction

Liver Biotech is a national high-tech enterprise specializing in the isolation, cryopreservation and regeneration of primary hepatocytes. Tree Shrew Primary Hepatocytes are isolated from laboratory-bred tree shrews (Tupaia belangeri) using our proprietary high-efficiency cryopreservation technology. These cells undergo standardized and large-scale isolation and cryopreservation processes, ensuring consistent quality. Post-thawing, the hepatocytes exhibit adhesion capability and are suitable for applications such as hepatitis virus (HBV/HCV) research and drug development.

.II Reagents and Materials

- Primary tuoaia hepatocytes (Cat# LV-PTH001/2)
- Recovery Medium (for adherent hepatocytes, Cat# LV-Rec001)
- Purification Medium (provided free with cells if required)
- Plating Medium (for adherent hepatocytes, Cat# LV-WEP003)
- Maintenance Medium (for adherent hepatocytes, Cat# LV-WEM003)
- Collagen-Coated Plates (for adherent hepatocytes, Cat# LV-Coated)
- William's Medium E (WE) (for suspension hepatocytes, customer-supplied)
- Non-TC Treated Plates (for suspension hepatocytes, customer-supplied)
- Sterile 15mL centrifuge tubes
- Disposable pipettes
- Wide-bore pipette tips (standard tips modified by trimming the ends followed by sterilization)
- Pipettors
- Constant temperature water bath (pre-warmed to 37℃, calibration with thermometer required)
- Refrigerated centrifuge (equipped with swing-out rotor compatible with 15mL tubes)
- Biological safety cabinet

Critical: Always use liquid nitrogen for cell transfer and wide-bore tips throughout the procedure.



III Thawing of Suspension Cells

1. In the biosafety cabinet, add 10 mL of WE medium to a 15 mL centrifuge tube. Pre-warm the tube in a 37°C water bath for 20 minutes, then transfer back to the biosafety cabinet for use. Keep purification medium on ice or at 4°C (if required). Pre-warm WE medium to 37°C.
2. Quickly transfer the cryopreserved hepatocytes from liquid nitrogen to a 37°C water bath. Submerge the cryovial as much as possible (keeping the cap above the water surface) and swirl gently in wide clockwise circles.
3. Thaw the cryovial for approximately 90–120 seconds, until only small ice crystals remain.
4. Disinfect the outer of the cryovial with 75% ethanol, then transfer it into the biosafety cabinet.
5. Using a wide-bore pipette tip, gently aspirate the cell suspension and transfer them dropwise into the 10 mL pre-warmed WE medium (Note: Residual cells in the cryovial and pipette tip can be recovered by rinsing with 1 mL WE medium, which should then be added to the same centrifuge tube). Mix gently by inverting the tube 2–3 times.
6. Centrifuge at 50 × g for 5 minutes. Discard the supernatant and resuspend the cell pellet in WE medium. Determine cell viability using Trypan Blue exclusion (mix the cell suspension with 0.4% Trypan Blue at a 9:1 ratio, and manually count viable cells using a hemocytometer (automated counters are not recommended for viability assessment). Total cell density can be determined using an automated counter. Perform total

cell counting in triplicate and calculate the average; cell viability can be assessed in a single measurement. Adjust seeding density and drug concentration based on experimental requirements. For assays evaluating the metabolic function of suspension cells, a seeding density of 1 × 10^6 cells/mL in 12- or 24-well plates (1 mL/well) is recommended (refer to group standard T/CSCB 0008-2021).

7. If cell viability is below 70%, use purification medium to remove dead cells: Centrifuge the cell suspension at 100 × g for 5 minutes at room temperature, discard the supernatant, and resuspend the pellet in 2 mL purification medium (provided free of charge). Carefully overlay 1 mL WE medium along the inner wall of the tube to form a sharp interface, taking care not to disturb the lower layer.. Centrifuge at 800×g for 10 minutes at 4°C with an acceleration setting of 9 and a deceleration setting of 1. After centrifugation, carefully aspirate the cloudy interphase (viable cell layer) and transfer it to a new 15 mL centrifuge tube. Add 2× volumes of WE medium, mix gently, and centrifuge at 50×g for 5 minutes at room temperature. Discard the supernatant, resuspend the pellet in 2 mL WE medium, and proceed with counting and seeding as described in Step 6.

IV Cells Thawing and Plating of Adherent Cells

1. In the biosafety cabinet, add 10 mL of recovery medium (Cat# LV-Rec001) to a 15 mL centrifuge tube. Pre-warm in a 37℃ water bath for 20 minutes, then transferring back to the biosafety cabinet. Keep purification medium on ice or at 4°C.. Pre-warm plating medium to 37℃.
2. Quickly transfer cryopreserved hepatocytes from liquid nitrogen to a 37℃water bath. Submerge the cryovial as much as possible while keeping the cap above the water surface, and swirl gently in wide clockwise circles.
3. Thaw the cryovial for approximately 90-120 seconds until only small ice crystals remain.
4. Disinfect the cryovial with 75% ethanol and transfer it into the biosafety cabinet.
5. Using a wide-bore pipette tip, gently aspirate the cell suspension and transfer it dropwise into 10 mL pre-warmed recovery medium. (Note: Residual cells in the cryovial and pipette tip can be recovered by rinsing with 1 mL recovery medium, which should then be added to the same centrifuge tube. Mix gently by inverting the tube 2–3 times.
6. Centrifuge at 50×g for 5 minutes. Discard the supernatant and resuspend the cell pellet in plating medium. Assess cell viability using Trypan Blue exclusion: mix the cell suspension with 0.4% Trypan Blue at a 9:1 ratio, and manually count viable cells using a hemocytometer. Automated counters are not recommended for viability assessment, although total cell count can be determined using an automated counter. Perform total cell count in triplicate and calculate the average; viability assessment can be performed in a single replicate..
7. If cell viability is >70%, seed the cells at 1.2-1.5×10^5 cells/cm^2 onto collagen-coated plates. Gently swirl the plate and incubate at 37℃ under 5% CO_2 for 16 hours.
8. If cell viability is <70%, perform cell purification: Centrifuge the cell suspension at 100×g for 5 minutes, discard the supernatant, and resuspend the pellet in 2 mL purification medium (provided free of charge). Carefully overlay 1 mL plating medium along the inner wall of the tube to form a distinct interface, taking care not to disrupt the lower layer. Centrifuge at 800×g for 10 minutes with an acceleration setting of 9 and a deceleration setting of 1. After centrifugation, carefully aspirate the cloudy interphase (viable cell layer) and transfer it to a new 15 mL centrifuge tube. Add 2× volumes of plating medium, mix gently, and centrifuge at 50 × g for 5 minutes at room temperature. Discard the supernatant, resuspend the pellet in 4 mL plating medium, and proceed with cell counting and plating as described in Steps 6 and 7.

Important Notes:

-For optimal cell attachment, ensure even cell distribution to avoid visible cell aggregation, which may impair adhesion.

-Recommendation 1: After mixing the cell suspension, dispense it into the culture wells without swirling. Allow cells to settle undisturbed in the biosafety cabinet for 10 minutes to ensure uniform attachment before transferring the plate to the incubator.

-Recommendation 2: For 48- or 96-well plates, increase the medium volume by 1.5–2× to counteract edge tension effects. Let the plate stand in the biosafety cabinet for 10 minutes before transferring to the incubator to prevent uneven cell distribution (edge accumulation).

V. Hepatocyte Maintenance and HBV Infection

1. After 16 hours of seeding, cells should be fully adherent. Aspirate the plating medium (containing dead cells) and replace it with maintenance medium. Refresh the medium every 2 days. (Note: For improved culture cleanliness, cells may be washed with WE medium; do not use PBS.)
2. Prepare infection medium: Add 4% PEG8000 to maintenance medium and mix thoroughly. Use HBV virus (HepAD38 at MOI = 500-1000; purified patient serum at MOI = 100-500; higher MOI may improve infection efficiency).
3. Aspirate the old medium and add the prepared infection medium. Incubate cells at 37℃ with 5% $CO_2$for 16 hours or

overnight.

4. After infection, wash cells three times with maintenance medium, then add fresh maintenance medium and continue culture at 37℃ with 5% CO_2.

5. Collect supernatant every 2 days post-infection for viral antigen and HBV DNA detection. Harvest supernatants at 3, 5, 7, and 9 days post-infection (dpi). Terminate the culture at 9 dpi, or extend the culture duration based on cell condition.

VI After-Sales Service

If you find any quality issues with the product, please collect the original data and contact the our sales or technical support immediately. We will assign dedicated personnel to ensure after-sales service. Each laboratory has different conditions, operational protocols, and user proficiency may contribute to experimental failures due to objective factors. If the instructions are not strictly followed, or beyond the after-sales time, we will not provide after-sales service. We appreciate your understanding and support.

After-sales validity period and required original data:

-Thawing issues: Report abnormalities immediately and provide Trypan Blue or AO/PI staining results.

-Contamination issues: Contamination is found within 96 hours; provide phase-contrast microscope images.

-Purity issues: Within 1 month (IF/flow cytometry data)

Tel: 0755-28284050

Technical Support: 19902901483 (Dr. Zhou)