

# User Guide: Thawing and Plating Cryopreserved Liver Sinusoidal Endothelial Cells

## Product Information

Liver Sinusoidal Endothelial Cells (LSECs), cryopreserved immediately after isolation (P0), serve as a key model to bridge physiology and pathology. Their core functions, including anti-inflammatory activity, endocytosis, pro-angiogenic secretion, and HSC maintenance, make them directly relevant for studying NAFLD, AFL, HCC, regeneration, and fibrosis. Identity is verified by markers such as CD146. In culture, they readily form a cobblestone monolayer, offering a physiologically relevant system for disease modeling. For optimal performance, it is recommended to use the cells with their dedicated medium kits: Liver Sinusoidal Endothelial Cell Thawing Medium Kit (LSECT-500K) and Liver Sinusoidal Endothelial Cell Maintenance Medium Kit (LSECM-500K).

Cat. No.	Product Description	Size (Cells)
CSD-LSEC-05	Cryopreserved SD Rat Liver Sinusoidal Endothelial Cells	0.5 million
CCD-LSEC-05	Cryopreserved CD-1 Mouse Liver Sinusoidal Endothelial Cells	0.5 million
CBD-LSEC-05	Cryopreserved Beagle Dog Liver Sinusoidal Endothelial Cells	0.5 million
CCY-LSEC-05	Cryopreserved Cynomolgus Monkey Liver Sinusoidal Endothelial Cells	0.5 million
CBM-LSEC-05	Cryopreserved Bama Minipig Liver Sinusoidal Endothelial Cells	0.5 million

## Storage & Shelf Life

Stable for 5 years at  $\leq -150^{\circ}\text{C}$ .

## Thawing Protocol

- The complete Liver Sinusoidal Endothelial Cell Thawing Medium and Maintenance Medium should be pre-warmed to  $37^{\circ}\text{C}$  before use. Prepare collagen I-coated plates. For detailed instructions, please refer to ***Liver Sinusoidal Endothelial Cell Culture Medium Kit Datasheet***.
- Transfer 15 mL of pre-warmed complete Liver Sinusoidal Endothelial Cell Thawing Medium to a sterile 50 mL centrifuge tube.
- Take cryovial out of the liquid nitrogen (transport on dry ice or in liquid nitrogen).
- Thaw cells for approx. 2 minutes at  $37^{\circ}\text{C}$  in the water bath. Remove the vial from the water bath while a small amount of ice remains to prevent over-thawing.
- Shake gently. When the cells pull away from the vial wall, transfer the content of vial into the complete Liver Sinusoidal Endothelial Cell Thawing Medium.
- Add 1 mL of complete Liver Sinusoidal Endothelial Cell Thawing Medium to the vial to wash any remaining cells from the vial(s).
- Spin down at  $300 \times g$  for 5 minutes at room temperature to pellet the Liver Sinusoidal Endothelial Cells.
- Carefully remove the supernatant without disturbing the pellet. Resuspend the cell pellet in 1 mL of complete Liver Sinusoidal Endothelial Cell Maintenance Medium.
- Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.
- Dilute the cells to the desired number of viable cells/mL (appropriate cell number is assay dependent- recommended  $5 \times 10^4$  -  $2 \times 10^5$  cells/cm<sup>2</sup>) with complete Liver Sinusoidal Endothelial Cell Maintenance Medium.
- Add an appropriate volume of diluted cells to collagen-coated cell culture plates as follows:
  - 6-Well plate: 2 mL/well (requires a total volume of 12 mL per 6-Well plate)
  - 12-Well plate: 1 mL/well (requires a total volume of 12 mL per 12-Well plate)
  - 24-Well plate: 0.5 mL/well (requires a total volume of 12 mL per 24-Well plate)
  - 48-Well plate: 0.2 mL/well (requires a total volume of 10 mL per 48-Well plate)
  - 96-Well plate: 0.1 mL/well (requires a total volume of 10 mL per 96-Well plate)

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12. Gently shake the plates in a back-and-forth and side-to-side manner to evenly distribute the cells. Avoid any circular movement, as this will cause the cells to unevenly pool in the center of the plates.
13. Carefully place the plates into a 37°C, 5% CO<sub>2</sub>, saturating humidity incubator to allow the cells to attach.
14. After 24h of cell adhesion, replace the pre-warmed complete Liver Sinusoidal Endothelial Cell maintenance Medium and proceed with the experiment. If necessary, replace the maintenance medium every 2-3 days.

## Related Products

Cat. No.	Product Description	Size	Store at
LSECT-500K	Liver Sinusoidal Endothelial Cell Thawing Medium Kit	500 mL/Kit	
LSECT-500b	Liver Sinusoidal Endothelial Cell Thawing Basal Medium (LSECTM-b)	500 mL	4°C
LSECT-500s	Liver Sinusoidal Endothelial Cell Thawing Supplement (LSECTM-s)	for 500 mL	-20°C
LSECM-500K	Liver Sinusoidal Endothelial Cell Maintenance Medium Kit	500 mL/Kit	
LSECM-500b	Liver Sinusoidal Endothelial Cell Maintenance Basal Medium (LSECMM-b)	500 mL	4°C
LSECM-500s- I	Liver Sinusoidal Endothelial Cell Maintenance Supplement I (LSECMM-s I)	for 500 mL	-20°C
LSECM-500s- II	Liver Sinusoidal Endothelial Cell Maintenance Supplement II (LSECMM-s II)	for 500 mL	-20°C
Col-Coated-6w	Collagen I Coated 6-Well Plate	1 Plate	4°C
Col-Coated-12w	Collagen I Coated 12-Well Plate	1 Plate	4°C
Col-Coated-48w	Collagen I Coated 48-Well Plate	1 Plate	4°C
Col-Coated-96w	Collagen I Coated 96-Well Plate	1 Plate	4°C
Col-Coated-384w	Collagen I Coated 384-Well Plate	1 Plate	4°C