User Guide:

Thawing and Plating Cryopreserved Hepatic Stellate Cells

Product Information

Hepatic stellate cells (HSCs) are liver-specific mesenchymal cells that play critical roles in liver physiology, tissue remodeling, and fibrogenesis. MileCell offers high-quality hepatic stellate cells (HSCs), available in cryopreserved format. Cell purity is assessed by Immunofluorescence analysis of α-smooth muscle actin expression. These ready-to-use cryopreserved cells are ideal for a range of applications, including liver fibrosis research, inflammatory signaling studies, and investigations into cellular activation mechanisms. In culture, the cells attach to uncoated or extracellular matrix-coated plates and can be activated to a myofibroblast-like phenotype for disease modeling. For optimal performance, it is recommended to use the cells with their dedicated medium kits: Hepatic Stellate Cell Thawing Medium Kit (HSCT-500K), Hepatic Stellate Cell Plating Medium Kit (HSCP-500K), Hepatic Stellate Cell Maintenance Medium Kit (HSCM-500K).

Cat. No.	Product Description	Size (Cells)
CHu-HSC-05	Human Hepatic Stellate Cells (P1)	0.5 million
CHu-HSC-05	Human Hepatic Stellate Cells (P2)	0.5 million
CSD-HSC-05	SD Rat Hepatic Stellate Cells	0.5 million
CCD-HSC-05	CD-1 Mouse Hepatic Stellate Cells	0.5 million
CCB-HSC-05	C57BL/6N Mouse Hepatic Stellate Cells	0.5 million
CBD-HSC-05	Beagle Dog Hepatic Stellate Cells	0.5 million
CCY-HSC-05	Cynomolgus Monkey Hepatic Stellate Cells	0.5 million
CBM-HSC-05	Bama Minipig Hepatic Stellate Cells	0.5 million

*Animal Hepatic Stellate Cells are cryopreserved immediately after isolation (P0).

Storage & Shelf Life

Stable for 5 years at ≤ –150°C.

Thawing Protocol

1. The complete Hepatic Stellate Cell Thawing Medium, Plating Medium, and Maintenance Medium should be pre-warmed to 37°C before use. Prepare collagen I-coated plates. For detailed instructions, please refer to ***Hepatic Stellate Cell Culture Medium Kit Datasheet***.
2. Transfer 15 mL of pre-warmed complete Hepatic Stellate Cell Thawing Medium to a sterile 50 mL centrifuge tube.
3. Take cryovial out of the liquid nitrogen (transport on dry ice or in liquid nitrogen).
4. Thaw cells for approx. 2 minutes at 37°C in the water bath. Remove the vial from the water bath while a small amount of ice crystals remains to prevent over-thawing.
5. Shake gently. When the cells pull away from the vial wall, transfer the content of vial into the complete Hepatic Stellate Cell Thawing Medium.
6. Add 1mL of complete Hepatic Stellate Cell Thawing Medium to the vial to wash any remaining cells from the vial(s).
7. Spin down at 250 x g for 5 minutes at room temperature to pellet the Hepatic Stellate Cells.
8. Carefully remove the supernatant without disturbing the pellet. Resuspend the cell pellet in 1 mL complete Hepatic Stellate Cell Plating Medium.
9. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.

User Guide:

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10. Dilute the cells to the desired number of viable cells/mL (appropriate cell number is assay dependent- recommended 4-6x10^4 cells/cm^2) with complete Hepatic Stellate Cell Plating Medium.
11. Add an appropriate volume of diluted cells to Collagen I 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)
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_2, saturating humidity incubator to allow the cells to attach.
14. After 24h of cell adhesion, replace the pre-warmed complete Hepatic Stellate Cell maintenance medium and proceed with the experiment. If necessary, replace the medium every 2-3 days.

Subculturing Protocol

Important Note: Mouse HSCs are not recommended for passaging. Limited passaging (1-2 generations) is possible for Human, Rat, Dog, and Monkey HSCs.

1. Aspirate and discard the culture medium. Refer to Table 1 to add sterile 1× DPBS, gently swirl the plate to wash the cells, then aspirate and discard the DPBS.
2. Refer to Table 1 to add the corresponding volume of 0.25% Trypsin to the culture plate well. Ensure it covers the surface evenly before transferring the plate to a 37°C, CO_2 incubator for 3-5 minutes.
3. Monitor the cells under a microscope. Digestion is complete when the cells change from a stellate morphology to a rounded shape and detach easily upon gentle agitation of the plate.
4. Add a volume of Maintenance Medium equivalent to three times the volume of trypsin used to the culture plate well to terminate digestion. Gently pipette the solution to dislodge any remaining cells.
5. Collect the cell suspension and centrifuge at 250 × g for 5 minutes at room temperature. After centrifugation, carefully aspirate and discard the supernatant.
6. Resuspend the cell pellet in Hepatic Stellate Cell Plating Medium. Seed the cells at a 1:2 split ratio for limited passaging culture.
7. Allow 24 hours for the cells to adhere completely. Thereafter, replace the complete Hepatic Stellate Cell Maintenance Medium every 2-3 days.

Table 1

Culture Plate	Surface Area (cm^2/well)	DPBS (ml/well)	Trypsin (mL/well)
6-well plate	9.5	4	2.0
12-well plate	3.9	2	1.0
24-well plate	1.75	1	0.5
48-well plate	1.0	0.4	0.2
96-well plate	0.32	0.2	0.1

User Guide:

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Related Products

Cat. No.	Product Description	Size	Store at
HSCT-500K	Hepatic Stellate Cell Thawing Medium Kit	500 mL/Kit	
HSCT-500b	Hepatic Stellate Cell Thawing Basal Medium (HSCTM-b)	450 mL	4℃
HSCT-500s	Hepatic Stellate Cell Thawing Supplement (HSCTM-s)	for 500 mL	-20℃
HSCP-500K	Hepatic Stellate Cell Plating Medium Kit	500 mL/Kit	
HSCP-500b	Hepatic Stellate Cell Plating Basal Medium (HSCPM-b)	450 mL	4℃
HSCP-500s-Ⅰ	Hepatic Stellate Cell Plating Supplement Ⅰ (HSCPM-s Ⅰ)	for 500 mL	-20℃
HSCP-500s-Ⅱ	Hepatic Stellate Cell Plating Supplement Ⅱ (HSCPM-s Ⅱ)	for 500 mL	-20℃
HSCM-500K	Hepatic Stellate Cell Maintenance Medium Kit	500 mL/Kit	
HSCM-500b	Hepatic Stellate Cell Maintenance Basal Medium (HSCMM-b)	450 mL	4℃
HSCM-500s	Hepatic Stellate Cell Maintenance Supplement (HSCMM-s)	for 500 mL	-20℃
Col-Coated-6w	Collagen I Coated 6-Well Plate	1 Plate	4℃
Col-Coated-12w	Collagen I Coated 12-Well Plate	1 Plate	4℃
Col-Coated-24w	Collagen I Coated 24-Well Plate	1 Plate	4℃
Col-Coated-48w	Collagen I Coated 48-Well Plate	1 Plate	4℃
Col-Coated-96w	Collagen I Coated 96-Well Plate	1 Plate	4℃
Col-Coated-384w	Collagen I Coated 384-Well Plate	1 Plate	4℃