

User Guide: Thawing and Plating Cryopreserved Non-Parenchymal Cells Mix

Product Information

Non-Parenchymal Cells Mix (NPCs Mix), cryopreserved immediately after isolation (P0), serve as a key model to bridge physiology and pathology. “NPCs Mix” consists a mix of different types of liver non-parenchymal cells, including Kupffer cells, liver endothelial sinusoidal cells, stellate cells, etc. MileCell provides high-quality, functionally validated NPCs Mix for highly reliable and reproducible outcomes. For optimal performance, it is recommended to use the cells with their dedicated medium kits: NPCs Mix Thawing Medium Kit (NPCT-500K) and NPCs Mix Maintenance Medium Kit (NPCM-500K).

Cat. No.	Product Description	Size (Cells)
CHu-NPC-10	Human Non-Parenchymal Cells Mix	1 million
CSD-NPC-10	SD Rat NPCs Mix	1 million
CCD-NPC-10	CD-1 Mouse NPCs Mix	1 million
CCB-NPC-10	C57BL/6N Mouse NPCs Mix	1 million
CBD-NPC-10	Beagle Dog NPCs Mix	1 million
CCY-NPC-10	Cynomolgus Monkey NPCs Mix	1 million
CBM-NPC-10	Bama Minipig NPCs Mix	1 million

Storage & Shelf Life

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

Thawing Protocol

- The complete NPCs Mix Thawing Medium and Maintenance Medium should be pre-warmed to 37°C before use. Prepare collagen I-coated plates. For detailed instructions, please refer to ***NPCs Mix Culture Medium Kit Datasheet***.
- Transfer 15mL of pre-warmed complete NPCs Mix Thawing Medium to a sterile 50mL 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°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 NPCs Mix Thawing Medium.
- Add 1ml of complete NPCs Mix 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 Non-Parenchymal Cells Mix.
- Carefully remove the supernatant without disturbing the pellet. Resuspend the cell pellet in 1mL of complete NPCs Mix 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 2×10^5 cells/cm²) with complete NPCs Mix 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)
- Gently shake the plates in a back-and-forth and side-to-side manner to evenly distribute the cells. Avoid any circular movement, as

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- this will cause the cells to unevenly pool in the center of the plates.
- 13. Carefully place the plates into a 37°C, 5% CO₂, saturating humidity incubator to allow the cells to attach.
 - 14. After 24h of cell adhesion, replace the pre-warmed NPCs Mix 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
NPCT-500K	NPCs Mix Thawing Medium Kit	500 mL/Kit	
NPCT-500b	NPCs Mix Thawing Basal Medium (NPCTM-b)	500 mL	4°C
NPCT-500s	NPCs Mix Thawing Supplement (NPCTM-s)	for 500 mL	-20°C
NPCM-500K	NPCs Mix Maintenance Medium Kit	500 mL/Kit	
NPCM-500b	NPCs Mix Maintenance Basal Medium (NPCMM-b)	500 mL	4°C
NPCM-500s- I	NPCs Mix Maintenance Supplement I (NPCMM-s I)	for 500 mL	-20°C
NPCM-500s- II	NPCs Mix Maintenance Supplement II (NPCMM-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