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)
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 ≤ –150°C.

Thawing Protocol

1. 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***.
2. Transfer 15mL of pre-warmed complete NPCs Mix Thawing Medium to a sterile 50mL 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 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 NPCs Mix Thawing Medium.
6. Add 1ml of complete NPCs Mix Thawing Medium to the vial to wash any remaining cells from the vial(s).
7. Spin down at 300 x g for 5 minutes at room temperature to pellet the Non-Parenchymal Cells Mix.
8. Carefully remove the supernatant without disturbing the pellet. Resuspend the cell pellet in 1mL of complete NPCs Mix Maintenance Medium.
9. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.
10. Dilute the cells to the desired number of viable cells/mL (appropriate cell number is assay dependent- recommended $2x10^5$ cells/cm^2) with complete NPCs Mix Maintenance Medium.
11. 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)
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.

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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 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℃
NPCT-500s	NPCs Mix Thawing Supplement (NPCTM-s)	for 500 mL	-20℃
NPCM-500K	NPCs Mix Maintenance Medium Kit	500 mL/Kit	
NPCM-500b	NPCs Mix Maintenance Basal Medium (NPCMM-b)	500 mL	4℃
NPCM-500s-Ⅰ	NPCs Mix Maintenance Supplement Ⅰ (NPCMM-s Ⅰ)	for 500 mL	-20℃
NPCM-500s-Ⅱ	NPCs Mix Maintenance Supplement Ⅱ (NPCMM-s Ⅱ)	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-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℃

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