

Thawing and culturing of cryopreserved primary non-parenchymal cells (NPC) in 2D

Version 03

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Note: This protocol describes the thawing of NPC from rat, mouse, human and marmoset monkey.

Required and recommended media and consumables

- Thawing and Plating Kit (PTK-NPC) consists of:
 - Thawing Buffer (TB)
 - NPC-Medium + separate supplements
- Collagen coated cell culture plates (not included in this kit, recommended for most NPC-cell types, not necessary for KC)

1. Arrival of the cryopreserved cells in your laboratory

- Place the cryogenic vial with frozen NPC immediately into the gas phase of a liquid nitrogen tank or in a freezer at a temperature below -135 °C.

2. Thawing and plating of primary NPC

This protocol describes the thawing of a single vial.

1. Warm water bath to 37 °C.
2. Before first use: For preparation of the ready-to-use NPC-Medium thaw the supplement vial at RT and add the vial content to the NPC-Medium. The ready-to-use NPC-Medium has then a shelf life of one month.
3. If necessary, cool centrifuge tubes at least 10 min on ice. Refer to the table below for the temperature of the media and tubes:

Cell type	Temp. TB	Temp. Centrifugation	Temp. Plating Medium	Notes
KC	4 °C	4 °C	4 °C	Work on ice!
SC	4 °C	4 °C	37 °C	Work at RT
LSEC	37 °C	20 °C	37 °C	Work at RT
NPC mix	4 °C	4 °C	4 °C	Work on ice!

4. Remove the vial with cells from liquid nitrogen storage/-150 °C and place it immediately into the 37 °C warm water bath until only a small bit of ice is visible (approx. 1-2 min).
5. Spray 70 % ethanol on the cryogenic vial for disinfection.
6. Dropwise add 1 mL TB to the cryogenic vial.
7. Transfer the cell suspension to a centrifuge tube and quickly add 3 mL TB.
8. Wash the cryogenic vial with 1 mL TB twice to remove the cells completely and combine it with the cells in the tube.

9. Gently swirl the tube slowly two or three times.
10. Centrifuge the cells: 300 x g, 8 min, temperature in table above
11. Remove the supernatant, then gently loosen the cells without adding any medium by gently agitating the bottom of the tube. Do not vortex or shake the cells.
12. Resuspend the pellet with 1-2 mL NPC-Medium for plating (see data sheet for post-thaw yield per vial).
13. Determine cell viability and live cell number with the trypan blue exclusion test in a counting chamber (Neubauer or equivalent counting chamber). Do not use automated cell counter.
14. Adjust cell suspension to the desired density for plating with required amount of NPC-Medium
 - The actual seeding density may vary and is therefore noted in the CoA
 - Do not add less than 0.5 mL cell suspension per well in 24-well plate
15. Plate the cells on a collagen-coated 24-well culture plate (not necessary for KC).
16. Incubate the cells at 37 °C and 5 % CO₂.

3. Culture of primary NPC

- After approx. 24 h: change medium to remove debris and dead/non-attached cells and use recommended PRIMACYT maintenance medium (see table below)
- Heat culture medium and PBS (optional) to 37 °C (no longer than 15 min).
- Recommended if cells adhered well: Wash the cells with warm PBS (1-2 times).
- Add 0.5 mL fresh culture medium per well.
- Change the medium at least every two to three days.
- Change the medium quickly, do not let the cells dry.

Cell type	Recommended Culture Medium (Maintenance Medium)
KC	MMKC
SC	MMNPC
LSEC	MMEC
NPC mix	MMNPC

FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURES.