MMI Protocol

Sample Lysis in EVO 96 Plate



Ice-Assisted Transfer of Tissue Sections and Single Cells

Introduction

The following protocol describes the ice-assisted transfer of tissue sections or single cells isolated using the CellCut HT laser microdissection system into a proteoCHIP EVO 96 plate.

Principle of Ice-Assisted Transfer

For the ice-assisted transfer, tissue sections or single cells are frozen together with either water or lysis buffer. Upon freezing, ice formation leads to the establishment of strong hydrogen bonds between the ice and the water-containing tissue. As a result, the tissue adheres much more strongly to the ice than to the silicone membrane, allowing it to be completely detached.

Note: The ice-assisted transfer works reliably with bidistilled water. The water can subsequently be removed by evaporation, allowing the desired volume of lysis buffer to be added. The direct use of a specific lysis buffer for the ice-assisted transfer should be tested beforehand.

Materials

- MMI 96-well isolation plate
  Part No.: 50400
- MMI flat metal pressure plate
- proteoCHIP EVO 96 plate (SCIENION)
  Part No.: C-PEVO-96-10
- Silicone sealing mat (nerbe plus GmbH)
  Part No.: 04-090-0000
- Bidistilled water or lysis buffer (composition depending on your downstream application)
- Laboratory freezer (-20 °C)
- 4 clamps (for uniform pressure during freezing)

Method

ROIs Transfer to the ProteoCHIP EVO 96 Plate

1. Collect tissue or cells into the MMI 96-well Isolation Plate using the MMI CellCut HT.
2. Add 13 µL of bidistilled water or lysis buffer into each target well of the EVO 96 plate.
3. Carefully place the MMI 96 Isolation Plate onto the EVO 96 plate, ensuring that the wells are precisely aligned so that the center of each well in the MMI Isolation Plate is directly above the center of the corresponding well in the EVO 96 plate.
4. Place the silicone sealing mat on top of the MMI Isolation Plate with the silicone plugs facing downward toward the wells. Ensure that each plug is centered over the corresponding well and fully engages the well opening.
5. Place the supplied flat MMI metal pressure plate on top of the silicone sealing mat to ensure even pressure distribution across all wells.
6. Secure the complete assembly using the supplied clamps. Verify that uniform pressure is applied across the entire plate area and that both plates remain fully aligned.
7. Place the assembly in a −20 °C freezer for at least 1.5 hours, or until all liquid in the EVO 96 wells is completely frozen.
8. Remove the assembly from the freezer and separate the MMI Isolation Plate from the EVO 96 plate immediately. To avoid thawing of the frozen droplets, the separation should be completed within 30 seconds after removal from the freezer.

Recommendations

- Avoid any lateral movement between the plates once they are brought into contact.
- Keep the time between removal from the freezer and plate separation as short as possible to prevent thawing.
- After successful transfer, the water can be evaporated and replaced with the appropriate lysis buffer for downstream sample preparation.
- Ensure that the freezer temperature is at least as low as −20 °C. If the temperature is higher, the storage time must be extended.

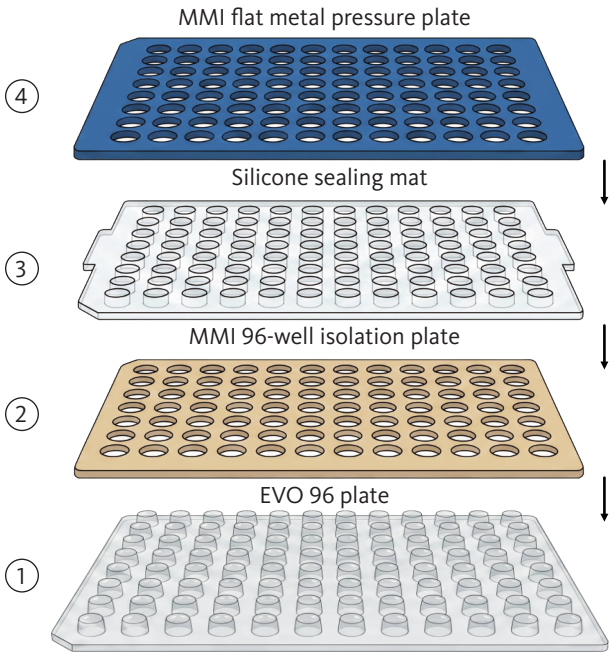


Fig. 1: Positioning of the plates for transfer to the EVO 96 plate using the ice-assisted transfer.

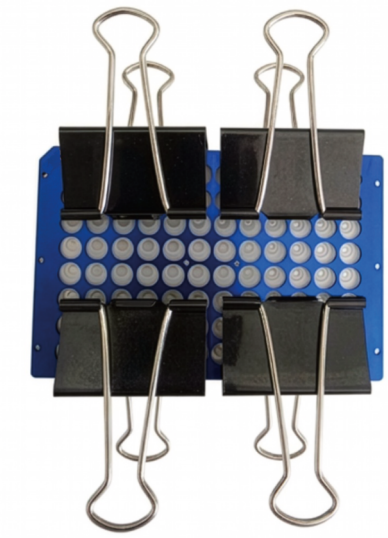


Fig. 2: The plates are pressed together using clamps to ensure that they remain firmly aligned. The clamped plate assembly is then placed in the freezer.

Verification of Transfer Efficiency

- Transfer efficiency can be verified by placing the MMI isolation plate under a microscope after separation.
- Successful transfer is indicated by the presence of the laser ablation mark while no tissue or cell material remains at the collection site.
- If residual tissue is still visible at the collection site, the transfer can be repeated before proceeding with downstream sample preparation.

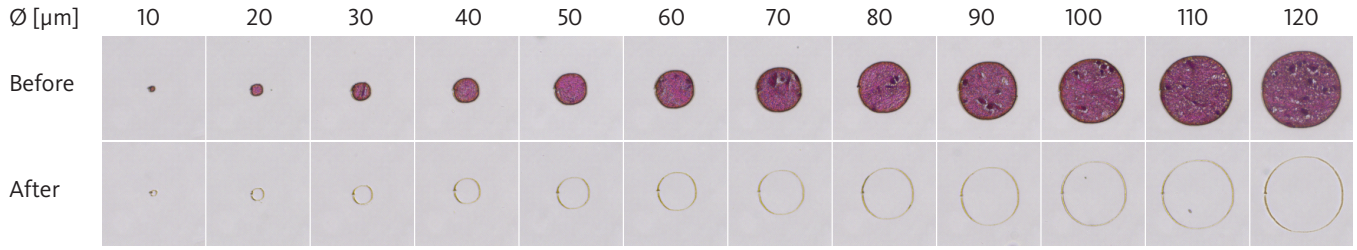


Fig. 3: Transfer efficiency of isolated tissue samples: After collection, all isolated tissue sections (10–120 µm in diameter) were successfully transferred. (FFPE tissue, 40× magnification)



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