User-developed protocol

User-Developed Protocol:

Concentration of RNA in 96-well plates using the MinElute® 96 UF PCR Purification Kit

This procedure has been adapted by customers and is for concentration of RNA in 96-well plates using the MinElute 96 UF PCR Purification Kit. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the *MinElute® 96 UF PCR Purification Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Equipment and reagents to be supplied by the user

- Multichannel pipet and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Disposable gloves
- Vacuum manifold (e.g., QIAvac Multiwell, cat. no. 9014579)
- Vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN Vacuum Pump)
- Vacuum Regulator (QIAGEN cat. no. 19530; for easy monitoring of vacuum pressures and easy releasing of vacuum).

Things to do before starting

- If using a microplate shaker for RNA elution in step 9, calibrate the shaker using the following steps:
    - Use a 96-well polystyrene microplate with 300 µl round-bottom wells.
    - Add 200 µl of a colored aqueous solution (e.g., bromophenol blue), to 2 wells.
    - Place the 96-well plate on the microplate shaker.
    - Set the speed to the lowest level and slowly increase the speed of the shaker. Ensure that the plate is fixed securely on top of the shaker.
    - The recommended shaking speed for elution is the maximum speed at which no liquid is splashed out of the wells.

Important points before starting

- MinElute 96 UF PCR Purification Plates are not certified as RNase free. However, no RNA degradation has been detected after concentration using this protocol.

Procedure

1. **Prepare the vacuum manifold according to the supplier's instructions.**
   Place a waste tray inside the base of the manifold.
2. **Place the MinElute UF PCR Purification Plate on top of the vacuum manifold.**
3. **Pipet the RNA samples onto the MinElute 96 UF PCR Purification Plate.**
   Note: Processing sample volumes larger than 150 µl may lead to increased processing time.
4. **Apply a vacuum and maintain at –800 mbar for 10 minutes or until the wells are completely dry. Switch off vacuum source.**
5. **Carefully remove the MinElute 96 UF PCR Purification Plate from the vacuum manifold.**
6. **Carefully tap the MinElute 96 UF PCR Purification Plate on a stack of clean absorbent paper to remove any liquid that might remain on the bottom of the plate.**
7. **Add 20 µl elution buffer (supplied with the original RNA isolation kit) to each well.**
8. **Elute RNA according to step 8a or 8b.**

8a. **Shake the MinElute 96 UF PCR Purification Plate on a microplate shaker for 2 min at the recommended speed (see "Things to do before starting").**
   Note: Ensure that the MinElute 96 UF PCR Purification Plate is fixed securely on top of the shaker.

8b. **Alternatively, purified RNA may be dissolved by pipetting samples up and down 20 times.**

9. **Recover the concentrated RNA by pipetting the eluate out of each well.**
   For easier recovery of the eluates, the plate can be held at a slight angle.