Laboratory Operating Procedure

Isolation of RNA from formalin-fixed and paraffin-embedded tissue using the RNeasy Mini Kit

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Introduction

The RNeasy FFPE Kit enables the purification of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. In principle the purification procedure consists of 5 steps. Firstly, paraffin is removed from the sections and secondly the tissue is lysed and digested with proteinase K followed by heat treatment. Thirdly, the supernatant is incubated with DNase followed by addition of RBC buffer and ethanol. Afterwards, the supernatant is loaded to a RNeasy MinElute column on which the total RNA is bound. The bound DNA is washed in two centrifugation steps to remove residual contaminants. Finally, the RNA is eluted in buffer AE or Nuclease-free water. The RNA solution can be directly used for downstream processing or stored at -80°C.

Sample Material

Tissue sections from formalin-fixed, paraffin-embedded material

Equipment and Reagents

RNA extraction requires a nuclease-free environment. For RNA isolation a separate set of pipets should be used. In addition, only nuclease-free tubes, aerosol-free pipette tips and nuclease free water (DEPC-treated) should be used. During the entire procedure personal protection equipment consisting of lab coat, gloves and protective goggles should be used. For more information, consult the appropriate material safety data sheets (MSDSs), available from the manufacturer.

Equipment

- Microcentrifuge (for 1.5 ml and 2 ml tubes)
- Vortexer
- Laminar flow
- Pipettes (10µl, 100µl, 1000µl)
- Disposable gloves
- 1.5 ml and/or 2 ml tubes
- Sterile, DNase-free pipet tips (with aerosol barriers to prevent cross contamination)
- Thermal mixer, heating block or waterbath

Reagents

- RNeasy FFPE mini kit (Qiagen, cat. no. 73504)
- For deparaffinization: Deparaffinization solution (cat. no. 19093) or alternative reagent (e.g. hepane, xylene, limonene, CitriSolv)
- Ethanol (100%, undenatured)
- RNAse free water (DEPC-treated)

Literature

- *RNeasy FFPE Handbook (12/2014)*

Preparation of reagents supplied with the RNeasy FFPE Mini kit

Buffer RPE

Before first use add 4 volumes (44 ml) ethanol (96–100%) to the bottle containing 11 ml Buffer RPE concentrate.

DNase I stock solution

Prepare DNase I stock solution by dissolving the lyophilized DNase I in RNase-free water. Therefore inject with an RNase-free needle and syringe a volume of 550µl RNase-free water into the vial and mix gently.
For long-term storage of DNase I, remove the stock solution from the vial and divide it into single-use aliquots. These aliquots can be stored at -15 to -30°C for up to 9 month. Thawed aliquots can be stored at 2-8°C for up to 6 weeks.

Before getting started

- Equibrilate all buffers to room temperature and mix reconstituted Buffer RPE by shaking.
- Set one thermal mixer, heat block, or water bath to 56°C and the second to 80°C.

Procedure

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections up to 4 sections (max. 40 µm in total)
3. Place the sections in a 2 ml microcentrifuge tube
4. Add 160 µl (1-2 sections) or 320 µl (>2 sections) Deparaffinization Solution
    a. vortex vigorously for 10 s, and centrifuge briefly.
5. Incubate at **56°C for 3 min**, then allow to cool at room temperature.
6. Add 150 µl (1-2 sections) or 240 µl (>2 sections) Buffer PKD
    a. Mix by vortexing.
7. Centrifuge for 1 min at 11,000 x *g* (10,000 rpm).
8. Add 10 µl proteinase K to the lower, clear phase.
    a. Mix gently by pipetting up and down.
9. Incubate at **56°C for 15 min**, then at **80°C for 15 min** with continuous shaking.
10. Transfer the lower, uncolored phase into a new 2 ml microcentrifuge tube
11. Incubate on ice for 3 min.
12. Centrifuge for 15 min at 20,000 x *g* (13,500 rpm).
13. Transfer the **supernatant** to a new microcentrifuge tube
14. Add DNase Booster Buffer equivalent to a tenth of the total sample volume (approximately 16 µl (2 sections) or 25 µl (>2 sections)) and 10 µl DNase I stock solution.
    a. Mix by inverting the tube.
    b. Centrifuge briefly to collect residual liquid from the sides of the tube.
15. Incubate at room temperature for 15 min.
16. Add 320 µl (1-2 sections) or 500 µl (>2 sections) Buffer RBC
    a. Mix the lysate thoroughly.
17. Add 720 µl (1-2 sections) or 1200 µl (>2 sections) ethanol (100%) to the

sample.

a. Mix well by pipetting.

18. Transfer 700 µl of the sample, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube
19. Centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. → Repeat this step until the entire sample has passed through the RNeasy MinElute spin column.
20. Add 500 µl Buffer RPE to the RNeasy MinElute spin column.
21. Centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.
22. Add 500 µl Buffer RPE to the RNeasy MinElute spin column.
23. Centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm). Discard the collection tube with the flow-through.
24. Place the RNeasy MinElute spin column in a new 2 ml collection tube. Open the lid of the spin column.
25. Centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.
26. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube. Add 30 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.