Total RNA Extraction from Formalin-Fixed, Paraffin-Embedded (FFPE) Blocks

Zhihai Ma*

Department of Pathology, Stanford University School of Medicine, Stanford, USA
*For correspondence: zhihaima@stanford.edu

[Abstract] Total RNA is extracted from fixed biological specimens by this method with higher yield than commercial kits. The product contains intact micro RNAs and small RNAs, and fragmented long RNAs.

Materials and Reagents

1. Paraffin embedded specimen
2. 100% xylene (Thermo Fisher Scientific, catalog number: 6601)
3. 100% ethanol
4. Protease K (Life Technologies, Ambion®, catalog number: AM2546)
5. Trizol (Life Technologies, Invitrogen™, catalog number: 15596-018)
6. Chloroform (Sigma-Aldrich, catalog number: C2432)
7. Glycogen (F. Hoffmann-La Roche, catalog number: 10901393001)
8. Isopropyl alcohol (Isopropanol)
9. RNase-free water or TE buffer (USB, catalog number: 75834; Promega Corporation, catalog number: P1193)
10. Tris-HCl (Life Technologies, Invitrogen™, catalog number: 15568-025)
11. $CaCl_2$ (Sigma-Aldrich, catalog number: C5670)
12. Sodium dodecyl sulfate (Life Technologies, Invitrogen™, catalog number: 15525-017)
13. Commercial kit (Life Technologies, Ambion®, Austin, TX)
14. Sodium dodecyl sulfate
15. Protease digestion buffer (see Recipes)

Equipment

1. Microtome
2. Microcentrifuge
3. Siliconized tubes (Thomas Scientific, catalog number: 2591L12)

Procedure

1. Cut 20 µm sections from the interior of the paraffin block using a microtome, to minimize the nucleic acid damaged by exposure to the atmosphere during storage (for recovery of miRNA ≥20 µm slices are recommended, otherwise the miRNA will be lost during deparaffin washes).
2. Place tissue slices into 1.5 ml siliconized tubes, and add 1 ml 100% xylene to the sample.
3. Incubate at 50 °C for 3 min to melt the paraffin. Centrifuge the sample for 1 min at maximum speed to pellet the tissue, then discard the xylene without disturbing the pellet. Repeat the xylene wash once.
4. Wash the pellet twice with 1 ml 100% ethanol and air dry.
5. Add 150 µl 1x protease K digestion buffer containing 500 µg/ml protease K to each sample, incubate at 55 °C for 3 h.
6. Add 1 ml Trizol to each sample, incubate at 15 to 30 °C for at least 5 min to dissociate nucleoprotein complexes. Add 0.2 ml of chloroform, vortex the tubes vigorously for 15 sec and incubate at 15 °C to 30 °C for 2 to 3 min. Centrifuge the samples at no more than 12,000 *x g* for 15 min at 4 °C.
7. Transfer the aqueous phase to a fresh tube, add 10 µg glycogen and mix. Precipitate the total RNA by mixing with 0.6 ml isopropyl alcohol, and put the tube at -20 °C for at least 1 hr. Centrifuge at 12,000 *x g* for 10 min at 2-8 °C.
8. Wash the RNA pellet with 100% ethanol, briefly air-dry. Dissolve in RNase-free water or TE.

Notes

1. Most mature microRNAs and other small RNAs are intact, while most mRNA and other long RNAs are fragmented during formalin fixation. The yield is about 3 times higher than a commercial kit.
2. The RNA product is suitable for microRNA study or mRNA profiling by 3SEQ. Siliconized tube and glycogen are used to get higher yield of small RNAs.

Recipes

1. Protease digestion buffer
   20 mM Tris-HCl (pH 8.0)
   1 mM $CaCl_2$
   0.5% sodium dodecyl sulfate

Acknowledgments

This protocol was adapted from Ma *et al.* (2009).

References

1. Ma, Z., Lui, W. O., Fire, A. and Dadras, S. S. (2009). Profiling and discovery of novel miRNAs from formalin-fixed, paraffin-embedded melanoma and nodal specimens. *J Mol Diagn* 11(5): 420-429.

