

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

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**[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<sub>2</sub> (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  $\mu\text{m}$  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  $\geq 20$   $\mu\text{m}$  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  $\mu\text{l}$  1x protease K digestion buffer containing 500  $\mu\text{g/ml}$  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  $\times g$  for 15 min at 4 °C.
7. Transfer the aqueous phase to a fresh tube, add 10  $\mu\text{g}$  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  $\times g$  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  $\text{CaCl}_2$
  - 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.