Extraction of total RNA from fresh/frozen tissue (FT)

Protocol For:

Methods comparison for high-resolution transcriptional analysis of archival material on Affymetrix Plus 2.0 and Exon 1.0 microarrays

Kim M. Linton[1,5*], Yvonne Hey[2*], Sian Dibben[2], Crispin J. Miller[3], Anthony J. Freemont[4], John A. Radford[1,5], and Stuart D. Pepper[2]
[1]Cancer Research UK Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, UK, [2]Molecular Biology Core Facility, Cancer Research UK, Paterson Institute for Cancer Research, The University of Manchester, Manchester, UK, [3]Applied Computational Biology and Bioinformatics Group, Cancer Research UK, Paterson Institute for Cancer Research, The University of Manchester, Manchester, UK, [4]School of Clinical & Laboratory Sciences, The University of Manchester, Manchester, UK, and [5]School of Cancer and Imaging Sciences, The University of Manchester, Manchester, UK

**K.L. and Y.H. contributed equally to this work.*

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Protocol overview

• The extraction method (steps 2–21) is taken from the method supplied with TRIzol reagent (Invitrogen, Paisley, UK).

• Recover tumor tissue at the time of surgery, trim into 1-cm^3 fragments, and immerse immediately in TRIzol reagent prior to freezing at -80°C.

• Thaw and weigh tissue prior to RNA extraction, working quickly.

• Use a tissue power homogenizer (or a mortar and pestle) to homogenize tissue by hand.

• All centrifugation steps are carried out at 4°C.

Procedure

1. Prior to RNA extraction:
   a. Autoclave or wash equipment (i.e., tissue storage container, homogenizer blades, forceps, scalpel holder) in Neutracon solution for 2–4 h.
   b. Rinse equipment well in 1% SDS (prepared using DEPC-treated or other nuclease-free water).
   c. Rinse in 100% ethanol and leave to air-dry.
2. Weigh thawed sample to determine quantity of TRIzol reagent required (use 1 mL TRIzol per 50–100 mg of tissue).
3. Homogenize sample using tissue homogenizer.
4. Centrifuge at 12,000× *g* for 10 min.
5. Transfer cleared homogenate to fresh tube; discard insoluble material and upper fat layer, if present.
6. Incubate homogenized sample at room temperature for 5 min.
7. Add 0.2 mL chloroform per 1 mL TRIzol and cap tube tightly.
8. Shake vigorously by hand for 15 s.
9. Incubate at room temperature for 3 min.
10. Centrifuge at 12,000× *g* for 15 min.
11. Transfer aqueous phase (colorless upper phase) to a new tube.
12. Retain organic phase for DNA/protein extraction, if required (store at -20°C).
13. Add 0.5 mL isopropyl alcohol per 1 mL TRIzol.
14. Incubate at room temperature for 10 min.
15. Centrifuge at 12,000× *g* for 10 min (RNA forms a gel-like pellet on the side and bottom of the tube; discard the supernatant).
16. Add 1 mL 75% ethanol per 1 mL TRIzol and vortex for 10 s.
17. Centrifuge at 7500× *g* for 5 min.
18. Air-dry RNA pellet for 5–10 min.
19. Add 20 µL RNase-free water and mix by gentle pipetting.
20. Incubate at 60°C for 10 min.
21. Store RNA in labeled tube at -80°C until required.

Reagents

- TRIzol (Invitrogen)
- Neutracon (Decon, East Sussex, UK)
- Chloroform (Sigma-Aldrich, Poole, Dorset, UK)
- Ethyl alcohol (Sigma-Aldrich)
- Isopropyl alcohol (Sigma-Aldrich)
- DEPC-free water (Sigma-Aldrich)
- RNase-free water (Ambion, Huntingdon, Cambridgeshire, UK)

Equipment

- Tissue storage container
- Homogenizer blades
- Forceps
- Scalpel
- Scalpel holder

Address correspondence to Kim Linton, Cancer Research UK Department of Medical Oncology, The Christie NHS Foundation Trust, Wilmslow Road, Withington M20 4BX, Manchester, UK.
email: kim.linton@christie.nhs.uk