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Purification of RNA from cells and tissues by acid phenol–guanidinium thiocyanate–chloroform extraction

This protocol describes a single-step technique for the purification of RNA. Cells are homogenized in guanidinium thiocyanate and the RNA is purified from the lysate by extraction with phenol–chloroform at reduced pH¹. This method allows many samples to be processed simultaneously and quickly. The yield of total RNA depends on the tissue or cell source and is generally in the range of 4–7 µg per ml starting tissue or 5–10 µg per 10⁶ cells. All reagents used in this protocol must be prepared with diethyl pyrocarbonate (DEPC)-treated H₂O.

PROCEDURE

1| Prepare cells or tissue samples for isolation of RNA as appropriate for the material under study. Consult the table below for the amounts of solution D (4 M guanidinium thiocyanate, 25 mM sodium citrate•2H₂O, 0.5% (wt/vol) sodium lauryl sarcosinate, 0.1 M 2-mercaptoethanol) required for different types of samples.

Amount of tissue or cells	Amount of solution D
100 mg of tissue	3 ml
75-ml (T-75) flask of cells	3 ml
60-mm plate of cells	1 ml
90-mm plate of cells	2 ml

For tissues:

- (i) Isolate the desired tissues by dissection and place them immediately in liquid nitrogen.
- (ii) Transfer ~100 mg of the frozen tissue to a mortar containing liquid nitrogen and pulverize the tissue using a pestle. Keep the tissue frozen by the addition of liquid nitrogen.
- (iii) Transfer the powdered tissue to a polypropylene snap-cap tube containing 3 ml of solution D and homogenize at 15–25 °C for 15–30 s with a Polytron homogenizer (Kinematica).

For mammalian cells grown in suspension:

- (i) Harvest the cells by centrifugation at 200–1,900g at 15–25 °C for 5–10 min. Resuspend the cells in 1–2 ml of sterile ice-cold PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄).
- (ii) Harvest the cells again by centrifugation, remove the PBS completely by aspiration and add 2 ml of solution D per 10⁶ cells.
- (iii) Homogenize the cells with a Polytron homogenizer at 15–25 °C for 15–30 s.

For mammalian cells grown in monolayers:

- (i) Remove the medium and rinse the cells once with 5–10 ml of sterile ice-cold PBS.
- (ii) Remove PBS and lyse the cells in 2 ml of solution D per 90-mm culture dish (1 ml per 60-mm dish). Transfer the lysates to a polypropylene snap-cap tube.
- (iii) Homogenize the lysates with a Polytron homogenizer at 15–25 °C for 15–30 s.

2| Transfer the homogenate to a fresh tube and sequentially add 0.1 ml of 2 M sodium acetate (pH 4.0), 1 ml of phenol and 0.2 ml of 49:1 (wt/vol) chloroform/isoamyl alcohol per milliliter of solution D. After addition of each reagent, cap the tube and mix the contents thoroughly by inversion.

Processing of
cells or tissue
samples

Purification of RNA

- 3| Vortex the homogenate vigorously for 10 s. Incubate the tube for 15 min on ice.
- 4| Centrifuge the tube at 10,000g at 4 °C for 20 min and then transfer the upper, aqueous phase containing the extracted RNA to a fresh tube.
- 5| Add an equal volume of isopropanol to the extracted RNA. Mix the solution well and allow the RNA to precipitate at –20 °C for at least 1 h.
- 6| Collect the precipitated RNA by centrifugation at 10,000g at 4 °C for 30 min.
- 7| Carefully decant the isopropanol and dissolve the RNA pellet in 0.3 ml of solution D for every 1 ml of this solution used in Step 1.
- 8| Transfer the solution to a microcentrifuge tube, vortex it well and precipitate the RNA with 1 volume of isopropanol at –20 °C for 1 h or more.
- 9| Collect the precipitated RNA by centrifugation at maximum speed at 4 °C for 10 min in a microcentrifuge. Wash the pellet twice with 75% ethanol, centrifuge again and remove any remaining ethanol with a disposable pipette tip. Allow the pellet to air dry for a few minutes before dissolving it in 50–100 µl of DEPC-treated H₂O and storing at –70 °C.
- 10| Estimate the concentration of the RNA by measuring the absorbance at 260 nm of an aliquot of the final preparation.

SOURCE

This protocol was adapted from "Purification of RNA from Cells and Tissues by Acid Phenol-Guanidinium Thiocyanate-Chloroform Extraction," in *Molecular Cloning: A Laboratory Manual* (eds. Sambrook, J. & Russell, D.W.). 7.4–7.8 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, 2001; <http://www.cshlpress.com/link/molclon3.htm>).

1. Chomczynski, P. & Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156–159 (1987).

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