

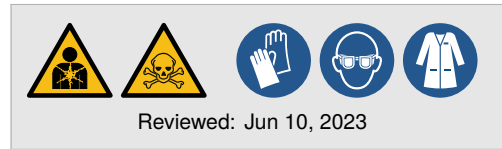
# Separation of RNA from DNA with guanidinium thiocyanate

Guanidinium thiocyanate extraction is a classical method for isolating high-quality RNA from cultured cells or tissue samples. It is particularly effective for recovering small RNAs and structured transcripts that may be underrepresented in column-based protocols.

This protocol follows the method of Chomczynski and Sacchi (1987), in which cells are lysed in a phenol-guanidinium solution and subjected to phase separation under acidic conditions. At low pH, DNA and proteins partition into the organic phase or interphase, while RNA remains soluble in the aqueous phase due to its increased polarity and base-pairing potential.

Compared to commercial kits, this method gives higher RNA yield and preserves a broader spectrum of transcript sizes. It is compatible with most cell and tissue types, but requires strict chemical safety precautions and additional handling steps.

*This is a bench card. Full protocol available online.*



## Procedures

### >> Cell homogenization and RNA extraction

- |   |   |
|---|---|
| <input type="checkbox"/> <a href="#">R0136 Solution D, 2 mL</a> | <input type="checkbox"/> <a href="#">R0141 Chloroform/Isoamyl alcohol</a> |
| <input type="checkbox"/> <a href="#">R0140 Phenol, 1 mL</a>     |   |

- (1.) Add 1 mL Solution D per 100 mg fresh tissue (minced on ice and homogenized) or  $1 \times 10^7$  cultured cells. Homogenize by pipetting up and down at least ten times.

**Critical:** Do not thaw frozen tissue. Pulverize tissue samples under liquid nitrogen before adding Solution D. Remove medium from suspension or adherent cells before lysis. ←

- (2.) Incubate for 5 min at room temperature.

**Critical:** Solution D inactivates RNases. Do not store samples in Solution D longer than 30 min; freeze if pausing. ←

- (3.) Add 0.1 vol 2 M sodium acetate (pH 4.0). Mix thoroughly by inversion.

- (4.) Add 1.0 vol water-saturated phenol. Mix thoroughly by inversion.

**Critical:** Use acidic phenol. Buffered phenol will not separate RNA from DNA and proteins. ←

- (5.) Add 0.2 vol chloroform/isoamyl alcohol (49:1). Shake vigorously for 15 s by hand. Do not vortex.

**Critical:** Ensure caps are tightly closed during mixing. ←

- (6.) Cool samples on ice for 15 min.

- (7.) Centrifuge at  $10\,000 \times g$  for 20 min at 4 °C.

- (8.) Carefully transfer the colorless aqueous layer to a new tube. Avoid disturbing the interphase. If turbid, repeat chloroform extraction. 📖

### >> RNA purification

- |  |  |
|--|--|
| <input type="checkbox"/> Isopropyl alcohol | <input type="checkbox"/> <a href="#">R0172 Water, 100 mL</a> |
| <input type="checkbox"/> 75% Ethanol       |  |

- (1.) Add an equal volume of isopropyl alcohol. Precipitate RNA on ice for 15–30 min.

- (2.) Centrifuge at  $12\,000 \times g$  for 15 min at 4 °C. Discard supernatant. Briefly spin again to remove residual solvent.

### *Separation of RNA from DNA with guanidinium thiocyanate*

- (3.) Resuspend pellet in 300  $\mu\text{L}$  Solution D. 📖
  - (4.) Add 300  $\mu\text{L}$  isopropyl alcohol. Repeat precipitation on ice.
  - (5.) Centrifuge at  $12\,000 \times g$  for 10 min at  $4\text{ }^\circ\text{C}$ . Discard supernatant.
  - (6.) Wash pellet with 600  $\mu\text{L}$  75% ethanol. Vortex briefly. Incubate 15 min at room temperature to remove guanidinium salts. 🕒 15 min
  - (7.) Centrifuge at  $12\,000 \times g$  for 10 min at  $4\text{ }^\circ\text{C}$ . Discard supernatant.
  - (8.) Air-dry pellet for 10–15 min.
- Critical:** Do not overdry or vacuum-dry RNA—solubility will decrease. ←
- (9.) Dissolve RNA in 100–200  $\mu\text{L}$  DEPC-treated water or 0.5% SDS. Incubate at  $60\text{ }^\circ\text{C}$  for 10–15 min.
  - (10.) Store RNA at  $-80\text{ }^\circ\text{C}$ . 🔧

### *List of references*

P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**(1), 156–159 (1987).

🔗 [Recipe \(available online\)](#) 🛠️ [Troubleshooting \(available online\)](#) 📄 [Notes \(available online\)](#)

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