

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.

Risk assessment

- **Chloroform is a KNOWN CARCINOGEN and REPRODUCTIVE TOXIN!**
- **Phenol is a REPRODUCTIVE TOXIN and causes serious chemical burns**
- **Guanidine salts form TOXIC GASES with bleach or strong acid**
- ▷ Work in a certified chemical fume hood
- ▷ Wear gloves, safety glasses, lab coat
- DO NOT add bleach or acidic solutions directly into sample waste



Reviewed: Jun 10, 2023

Procedures

>> Cell homogenization and RNA extraction

- Solution D, 2 mL *<R>*
- Phenol, 1 mL *<R>*
- Chloroform/Isoamyl alcohol *<R>*

- (1.) Add 1 mL Solution D per 100 mg fresh tissue (minced on ice and homogenized) or 1×10^7 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.

Note: If desired, recover genomic DNA from the interphase by back extraction.

>> **RNA purification**

<input type="checkbox"/> Isopropyl alcohol	<input type="checkbox"/> Water, 100 mL (R)
<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 × g for 15 min at 4 °C. Discard supernatant. Briefly spin again to remove residual solvent.
- (3.) Resuspend pellet in 300 μL Solution D.

This is why: A second precipitation improves purity at the cost of yield.

- (4.) Add 300 μL isopropyl alcohol. Repeat precipitation on ice.
- (5.) Centrifuge at 12 000 × g for 10 min at 4 °C. Discard supernatant.
- (6.) Wash pellet with 600 μL 75% ethanol. Vortex briefly. Incubate 15 min at room temperature to remove guanidinium salts. ⌚ 15 min
- (7.) Centrifuge at 12 000 × g for 10 min at 4 °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 μL DEPC-treated water or 0.5% SDS. Incubate at 60 °C for 10–15 min.
- (10.) Store RNA at –80 °C. ⌘

Analyses

- Measure absorbance at 260 nm, 280 nm, and 230 nm.

Nucleic acid	A260 = 1.0	A260/A280	A260/A230
ssRNA	40 ng/μL	1.8–2.0	2.0–2.2

Note: Purity ratios vary with base composition. A260/A280 shifts 0.2–0.3 with pH.

Critical: High A230 indicates residual guanidinium thiocyanate. ←

- Resolve 3 μg of RNA on a formaldehyde-agarose gel to assess 28S, 18S, and 5S rRNA integrity.

Troubleshooting

RNA purification

In Step 10:

- RNA degradation
 - Process or freeze tissue immediately after collection.
 - Use DEPC-treated water and store at –80 °C.
- DNA contamination
 - Use a larger volume of Solution D during homogenization.
 - Treat with DNase I prior to downstream analysis.

Recipes

Solution D

Amount	Ingredient	Stock	Final
100 g	Guanidine thiocyanate [593-84-0]	118.16 g/mol	4 M
7.0 mL	Sodium citrate, pH 7.0	0.75 M	25 mM
5.3 mL	Sodium <i>N</i> -lauroyl sarcosinate (Sarcosyl)	20%	0.5%
1.5 mL	2-Mercaptoethanol □ [60-24-2]	78.14 g/mol	100 mM
To 212 mL	Water, reagent-grade		

Prepare solution D in the manufacturer's bottle without weighing to avoid unnecessary handling of the hazardous material. Add 117 mL water per 100 g guanidinium thiocyanate (volume increase), bring to 65 °C to dissolve; dispense into 14 mL aliquots. Add 100 µL 2-mercaptoethanol per aliquot freshly before use. Stable for 3 months at room temperature.

Solution D

4 M Guanidine thiocyanate, 25 mM Sodium citrate, 0.5% Sarcosyl, □ 100 mM 2-Mercaptoethanol



Skin corrosion; Serious eye damage; Acute oral and inhalation toxicity; Germ cell mutagenicity; Hazardous to the aquatic environment

Expiry: Sign: R0136

Sodium *N*-lauroyl sarcosinate (Sarcosyl), 20%

Amount	Ingredient	Stock	Final
10.0 g	Sarcosyl [137-16-6]	293.38 g/mol	20%
To 50 mL	Water, reagent-grade		

Note: Sarcosyl does not precipitate and is often used in place of SDS.

20% Sodium *N*-lauroyl sarcosinate (Sarcosyl)



Eye irritation

Date: Sign: R0137

Sodium citrate, pH 7.0, 0.75 M

Amount	Ingredient	Stock	Final
2.65 g	Sodium citrate, tribasic, dihydrate [6134-04-3]	294.10 g/mol	0.75 M
50 µL	Sodium hydroxide (NaOH) ◇ R0048	1 M	
To 12 mL	Water, reagent-grade		

Stable for 1 year at room temperature.

0.75 M Sodium citrate pH 7.0



Expiry: Sign: R0138

Sodium acetate (NaOAc), pH 4.0, 2 M

Amount	Ingredient	Stock	Final
8 mL	Sodium acetate (NaOAc), pH 5.2 ◇ R0045	3 M	2 M
3–4 mL	Acetic acid, glacial [64-19-7]	17.4 M	
To 12 mL	Water, reagent-grade		

Stable for 1 year at room temperature.

2 M Sodium acetate (NaOAc) pH 4.0



Expiry: Sign: R0139

Phenol, water-saturated

Amount	Ingredient	Stock	Final
100 g	Phenol, nucleic acid grade [108-95-2]	94.11 g/mol	

Add 20 mL nuclease-free reagent-grade water, bring to 65 °C, let cool. Repeat until a second aqueous phase appears, then aspirate off the aqueous phase. Dispense into 10 mL aliquots, store at –20 °C. Stable for 1 month at 4 °C.

Phenol water-saturated		
		
Skin corrosion; Serious eye damage; Acute dermal, oral and inhalation toxicity; Germ cell mutagenicity		
Expiry:	Sign:	R0140

Chloroform/Isoamyl alcohol, 49:1

Amount	Ingredient	Stock	Final
49 mL	Chloroform [67-66-3]	119.38 g/mol	98%
1 mL	Isoamyl alcohol [123-51-3]	88.15 g/mol	2%

Prepare just before use. Store at 4 °C.

Chloroform/Isoamyl alcohol 98% Chloroform, 2% Isoamyl alcohol, 49:1		
		
Serious eye damage; Skin irritation; Acute inhalation toxicity; Carcinogenicity; Germ cell mutagenicity		
Date:	Sign:	R0141

Water, DEPC-treated

Amount	Ingredient	Stock	Final
0.1 mL	Diethyl pyrocarbonate (DEPC) [1609-47-8]	162.14 g/mol	0.1%
To 100 mL	Water, reagent-grade		

Water DEPC-treated		
Date:	Sign:	R0172

List of references

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

Change log

2023-06-10 Benjamin C. Buchmuller Adaptation as SOP.

Open Protocol — Part of the *Lab Protocols* collection (2025) by B. C. Buchmuller and contributors. This document is made available under the Creative Commons Attribution Share Alike 4.0 International License. To view a copy of this license, visit <https://creativecommons.org/licenses/by-sa/4.0/>.

For research use only. Provided in good faith, without warranty or liability for any use or results. Users are responsible for compliance with local regulations and institutional policies.

Current when printed. Visit <https://benjbuch.github.io/check/> or scan the QR code to check for updates.



2222e7b

