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.

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.

The method 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



In the event of EXPOSURE:
▷ **Rinse skin immediately with PEG 300 or PEG 400**
▷ **Flush affected eye(s) for 15 min**

Report to:
- **Principal Investigator/Supervisor**

Reviewed: Oct 27, 2026

Procedures

›› *Cell homogenization and RNA extraction*

☐ Solution D ⟋ R0136
☐ 2 M NaOAc, pH 4.0 ⟋ R0139
☐ Phenol, water-saturated ⟋ R0140
☐ Chloroform/Isoamyl alcohol, 49:1 ⟋ R0141

(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 × *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*

- ☐ Isopropyl alcohol
- ☐ 75% Ethanol, with DEPC-treated water
- ☐ 0.5% SDS, with DEPC-treated water (*optional*)
- ☐ DEPC-treated water ◇ R0172

(1.) Add an equal volume of isopropyl alcohol. Precipitate RNA on ice for 15–30 min 🗎 SOP 0021.

(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	A_{260} = 1.0	A_{260}/A_{280}	A_{260}/A_{230}
ssRNA	40 ng/µL	1.8–2.0	2.0–2.2

> ***Note:*** Purity ratios vary with base composition. A_{260}/A_{280} shifts by 0.2–0.3 with pH.

> ***Critical:*** High A_{230} 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.

Materials

Cell homogenization and RNA extraction

- ☐ **Solution D** ⟋ R0136
  Stable for 3 months at room temperature.
- ☐ **Sodium acetate** (**NaOAc**), 2 M, pH 4.0 ⟋ R0139
  Stable for 1 year at room temperature.
- ☐ **Phenol**, water-saturated ⟋ R0140
  Store at 4 °C. Alternatively, store at −20 °C. Stable for 1 month at room temperature.
- ☐ **Chloroform/Isoamyl alcohol**, 49:1 ⟋ R0141
  Store at 4 °C.

RNA purification

- ☐ **Isopropyl alcohol**
- ☐ **Ethanol**, 75%, with DEPC-treated water
- ☐ **SDS**, 0.5%, with DEPC-treated water (*optional*)
- ☐ **DEPC-treated water** ⟋ R0172

Recipes

Solution D

Amount	Ingredient		Stock	Final
100 g	Guanidine thiocyanate Skin corrosion (H314)	[593-84-0]	118.16 g/mol	4 M
7.0 mL	Sodium citrate, pH 7.0	[6132-04-3]	0.75 M	25 mM
5.3 mL	Sodium *N*-lauroyl sarcosinate (Sarcosyl) Serious eye damage (H318); Acute respiratory toxicity (H330)	[137-16-6]	20%	0.5%
1.5 mL	2-Mercaptoethanol ☐ Acute dermal toxicity (H310); Serious eye damage (H318); Reproductive toxicity (H361); Organ toxicity, repeated exposure (H373)	[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. Each 14 mL aliquot takes 100 µL 2-mercaptoethanol. Add 2-mercaptoethanol immediately 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



DANGER

Skin corrosion; Skin sensitization; Acute dermal toxicity; Organ damage through prolonged or repeated exposure; Reproductive toxicity; Acute inhalation toxicity; Acute oral toxicity; Aquatic toxicity (long-term)

- ☐ Collect as CYANIDE WASTE
- ☐ Keep alkaline, DO NOT acidify
- ☐ DO NOT wash into sewer

Date: Sign: R0136

Sodium N-lauroyl sarcosinate (Sarcosyl), 20%

Amount	Ingredient		Stock	Final
10.0 g	Sarcosyl Serious eye damage (H318); Acute respiratory toxicity (H330)	[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)



DANGER

Serious eye damage; Acute inhalation toxicity; Skin irritation

- ☐ Collect as HAZARDOUS WASTE
- ☐ DO NOT wash into sewer

Date: Sign: R0137

Sodium citrate, pH 7.0, 0.75 M

Amount	Ingredient		Stock	Final
2.65 g	Sodium citrate · 2 H_2O, tribasic [6132-04-3]		294.10 g/mol	0.75 M
50 µL	Sodium hydroxide (NaOH) Skin corrosion (H314); Serious eye damage (H318)	R0048	1 M	
To 12 mL	Water, reagent-grade			

Stable for 1 year at room temperature.

0.75 M Sodium citrate

pH 7.0

No GHS hazards at the indicated concentrations

- ☐ Hold for EHS review before disposal

Date: 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 Skin corrosion (H314)	[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



EPA Safer Choice low-concern

- ☐ Neutralise to pH 6–9
- ☐ Flush to drain with excess water

Date: Sign: R0139

Phenol, water-saturated

Amount	Ingredient		Stock	Final
100 g	Phenol, nucleic acid grade Skin corrosion (H314); Mutagenicity (H341); Organ toxicity, repeated exposure (H373)	[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 4 °C. Alternatively, store at −20 °C. Stable for 1 month at room temperature. ***Critical:*** Discard if the phenol has turned yellow or brown, oxidation products degrade RNA!

Phenol

water-saturated



Skin corrosion; Acute dermal toxicity; Acute inhalation toxicity; Acute oral toxicity; Germ cell mutagenicity; Organ damage through prolonged or repeated exposure; Serious eye irritation

- ☐ Collect as CORROSIVE WASTE
- ☐ Keep acids and bases separate

Date: Sign: R0140

Chloroform/Isoamyl alcohol, 49:1

Amount	Ingredient		Stock	Final
49 mL	Chloroform Carcinogenicity (H351); Reproductive toxicity (H361); Organ toxicity, repeated exposure (H372)	[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



Organ damage through prolonged or repeated exposure; Acute inhalation toxicity; Carcinogenicity; Reproductive toxicity; Skin and serious eye irritation; Acute oral toxicity

- ☐ Collect as HALOGENATED SOLVENT WASTE

Date: Sign: R0141

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, based on the cited Chomczynski & Sacchi (1987) chemistry; water-saturated phenol preparation (recipe R0140) adapted from Audrey L. Atkin "Preparation of Buffer Saturated Phenol for RNA Extractions" (University of Nebraska–Lincoln).

2026-10-27 **Benjamin C. Buchmuller** Narrowed the Atkin credit to the handling of phenol aliquots; corrected the sodium citrate dihydrate CAS number in recipe R0138. Follow sop_0021 for precipitation.

From the *Lab Protocols* collection by B. C. Buchmuller and contributors (2026). Licensed under Creative Commons Attribution Share Alike 4.0 International; see https://creativecommons.org/licenses/by-sa/4.0/ for the terms.

For research use. 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.

016c8c5