

Protocol for the isolation of RNA using GENAzol

1. Homogenization

Tissue

Homogenize tissue samples in 1mL of GENAzol per 50-100mg of tissue (max. 1/10 of the volume of GENAzol). For small quantities of tissue (1-10mg), add 800µL of GENAzol. For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed.

Plant tissue:

Following homogenization, insoluble material is removed by centrifugation at 12,000 x g for 10 minutes at +2°C to +8°C. Transfer the cleared homogenate to a fresh tube.

Cells Grown on Monolayer:

Lyse cells directly in a culture dish or flask by adding 1mL of GENAzol per 10cm² growth area (3.5cm diameter) after aspiration of cell culture medium. Pipette the cell lysate several times to ensure sufficient cell disruption.

Cells Grown in Suspension:

Pellet cells at 200 x g for 5 minutes at room temperature. Lyse cells with 1mL of GENAzol per 5 x 10⁶ cells (bacteria up to 1 x 10⁷) and pass the lysate several times through a pipette tip. For small quantities of cells (10²-10⁶), lyse cells in 800µL of GENAzol.

For **blood samples***, serum or other biological fluids add 750µL GENAzol per 250µL of sample volume.

* Biological fluids with high levels of protein or other contaminating substances (e.g., whole blood) may be diluted 1:1 with RNase-free, molecular biology grade water (M6340).

Note: At this stage, samples can be stored for at least one month at -70 °C.

2. Phase Separation

2a. Homogenize the sample by pipetting the suspension up and down several times.

2b. Incubate homogenized samples for 2 minutes at room temperature. This will improve separation of RNA from protein.

2c. Add 0.2 mL of chloroform per 1mL of GENAzol used.

2d. Cap tubes securely and shake vigorously by hand for 15 seconds.

2e. Incubate samples for 5-8 minutes at room temperature. This step will again improve the purity of RNA.

2f. Centrifuge samples at 12,000 x g for 15 minutes (or 2600 x g for 30 minutes) at +2°C to +8°C.

The sample will separate into a pale green, organic phase, an interphase, and a colorless upper aqueous phase.

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3. RNA Precipitation

3a. Transfer the aqueous phase very carefully, without disturbing the interphase to a new reaction tube.

3b. Add the same volume of cold isopropanol to precipitate the RNA. Keep on ice for 15 minutes. Keep the organic/interphase with the DNA/protein on ice, too.

3c. Centrifuge at 12,000 x g for 10 minutes (or 2600 x g for 30 minutes) at +2°C to +8°C.

4. RNA Wash

4a. Remove the supernatant (step 3c).

4b. Wash the pellet once with 75% ethanol, adding at least 1mL of ethanol per 1mL of GENAzol used.

4c. Vortex samples and centrifuge at 7500 x g for 5 minutes at +2°C to +8°C.

4d. Air dry the RNA pellet for 5-10 minutes (**NOTE: Do not vacuum centrifuge!**).

Note: At this stage, samples can be stored for one week at +2°C to +8°C, or 12 months at -20°C.

5. Re-dissolving the RNA

5a. Resuspend the pellet in 20 - 50µL PCR- or DEPC-treated water, 0.1mM EDTA solution by pipetting the solution up and down.

5b. Incubate for 10 minutes at 60°C if necessary.

Store RNA at -70 °C

Protocol for the isolation of DNA using GENAzol

After homogenization and phase separation the upper aqueous phase is removed for optional RNA precipitation, leaving the interphase and the organic phase for sequential isolation of DNA and protein.

Note: The interphase and organic phase can be stored overnight at +2°C to +8°C.

1. DNA Precipitation

- 1a. Remove any remaining aqueous phase overlying the interphase (step 3 of the RNA isolation protocol).
- 1b. Add 0.3mL of 100% ethanol per 1mL of GENAzol used and mix samples by repeated inversion of the reagent tube.
- 1c. Leave samples at room temperature for 3 - 5 minutes while mixing by inversion of the tube from time to time.
- 1d. Centrifuge at 2000 x g for 5 minutes at +2°C to +8°C.

Note: At this stage, samples can be stored for at least one month at +2°C to +8°C.

2. DNA Wash

- 2a. Remove the supernatant (phenol/ethanol) to waste or retain for protein isolation.
- 2b. Wash the DNA pellet, with 1mL of 0.1M sodium citrate in 10% ethanol per 1mL of GENAzol used.
- 2c. Incubate for 30 minutes at room temperature by mixing from time to time.
- 2d. Centrifuge samples at 2000 x g for 5 minutes at +2°C to +8°C.
- 2e. Repeat step 2c and 2d.

Note: Two washes are usually sufficient, however for large pellets containing >200µg of DNA an additional wash may be necessary.

3. Re-dissolving the DNA

- 3a. Air-dry the pellet for 15 minutes.
- 3b. Resuspend the pellet in in approx. 0.5mL 1X TE (pH8.0). Remaining cell fragments in this mixture are sedimented by centrifugation (12.000 x g/10 minutes), while the DNA stays in solution under these conditions. Transfer the DNA-containing supernatant to a new reaction tube.

Store DNA at -20°C

Protocol for the isolation of protein using GENAZol

1. Protein Precipitation

- 1a. To the retained supernatant (step 2a of the DNA isolation protocol) add 2mL of isopropanol per 1mL of GENAZol used.
- 1b. Incubate for 10 minutes at room temperature while mixing by hand from time to time.
- 1c. Centrifuge at 12000 x g for 10 minutes at room temperature.

2. Protein Wash

- 2a. Remove supernatant and wash the protein pellet twice.
- 2b. To wash the protein pellet, add 2mL of 0.3M guanidine hydrochloride in 95% ethanol per 1mL of GENAZol used.
- 2c. Incubate for 20 minutes at +2°C to +8°C.
- 2d. Centrifuge at 7500 x g for 5 minutes at +2°C to +8°C.
- 2e. Repeat step 2c and 2d twice.

Note: At this stage, samples can be stored for at least one month at +2°C to +8°C, or 12 months at -20°C.

3. Re-dissolving the Protein

- 3a. Air-dry or vacuum dry the protein pellet after removal of the supernatant for 5-10 minutes.
- 3b. Dissolve precipitate in 1% SDS by pipetting up and down. For difficult samples incubate at 50°C.
- 3c. Remove any insoluble material by an additional centrifugation at 10000 x g for 10 minutes at +2°C to +8°C
- 3d. Transfer the supernatant to another tube.

Store protein at -20°C.

References:

- 1.) Chomczynski, P. and Sacchi, N. Anal. Biochem. 162: 156-159. (1987)
- 2.) Chomczynski, P. BioTechniques 15: 532-537. (1993)
- 3.) Large and Small Scale Phenol Extraction. (Wallace, D.M. (1987) Methods Enzymol. 152, 33-41)

Trouble shooting

Problem	Possible Cause	Recommendation
	Interphase/organic phase pipetted up with aqueous phase	Do not attempt to draw off the entire aqueous layer after phase separation.
DNA contamination or RNA contamination	Insufficient removal of the aqueous phase from the organic phase	Remove remnants of the aqueous phase prior to DNA precipitation
	Insufficient wash of the DNA pellet	Make sure pellet is washed with 0.1M sodium citrate in 10% ethanol
Low RNA yield	Insufficient homogenisation or lysis of samples	Decrease the amount of starting material. Mince tissues into smaller pieces and make sure it is completely immersed in GENAZol for optimal lysis.
	Insufficient solubilisation of RA, DNA or protein.	Increase the solubilization by pipetting the sample repeatedly, heat the sample to 60°C.
	Loss of pellet	If starting sample is small, the pellet may not be easily visualized after precipitation, so care must be taken when removing the supernatant from the pellet.
DNA is degraded, RNA is degraded, or protein is degraded	Samples were not immediately processed or frozen after collection	Sample must be processed or frozen immediately after collection
	Isolated RNA, DNA or protein preparations were stored at the incorrect temperature	Store RNA samples at -80°C. Store DNA and protein samples at -20°C.
	RNase contamination	Protocol must be carried out carefully in a DNA-free, RNase-free environment. Addition of RNase Inhibitor to the extracted RNA sample can help prevent degradation of the sample.
Low A260/280 for RNA	Insufficient volume of GENAZol	Ensure that 1mL GENAZol per 10cm ² area of cells or 5 x 10E6 cells is used. If problem persists, increase GENAZol volume by 1.5x
	Contamination of interphase layer during separation of the RNA-containing aqueous layer	Pipette off the aqueous phase very carefully. It is important that none of the white interphase is transferred into your RNA sample, so we recommend that you leave the lower part of the aqueous phase intact
Low A260/280 for DNA	Phenol was not sufficiently removed from the DNA preparation	Wash the DNA pellet one additional time in 0.1M sodium citrate in 10% ethanol.

Storage and Stability:

GENAZol is shipped at room temperature. For optimal performance, we recommend storing at +2°C to +8°C.

Expiry:

When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.

Safety Precautions:

Toxic in contact with skin. Toxic if swallowed. Causes burns.

Please refer to the material safety data sheet for information regarding hazards and safe handling practice.

Labels:

GHS08



GHS06



GHS05

Signal word**DANGER****Usage**

For research usage only!

Not for human or animal diagnostic or therapeutic usage/applications. Not for consumption.