TRIzol Reagent

General Information

TRIzol is a complete, ready-to-use reagent for the isolation of RNA (as well as DNA and proteins) from cell and tissue samples of human, animal, plant, yeast, or bacterial origin, within one hour. TRIzol is a clear, red, monophasic solution of phenol and guanidinium isothiocyanate that simultaneously solubilizes biological material and denatures protein. This reagent performs well with both small and large quantities of tissue or cells.

1 mL of TRIzol is sufficient to isolate RNA and DNA from 1 × 107 cells or 100 mg of tissue.

Storage/Stability

TRIzol is shipped in a polypropylene bottle at ambient temperature. For optimal performance, we recommend to store at +4 °C. TRIzol is stable for 2 years.

Safety Precautions

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

Notes: Research Use Only.

Signal Word: DANGER

Features

- Quick isolation of high-quality total RNA, DNA and/or protein from a single sample
- Performs well with large or small amounts of tissue or cells
- Ready-to-use solution

Applications

- Purified RNA is ideal for any downstream application such as RT-PCR, in vitro translation, northern blotting, RNase protection assays or dot blot hybridization
- Purified DNA can be used for PCR and Southern blotting
- Purified protein can be used for western blotting

Required Materials Not Supplied

For RNA Isolation

Chloroform
Isopropyl alcohol
Ethanol, 70%
Nuclease-free water

For DNA Isolation

Ethanol
0.1 M sodium citrate, 10% ethanol
Ethanol, 75%
8 mM NaOH

For Protein Isolation

Ethanol
Isopropyl alcohol
0.3 M guanidine HCl in 95% ethanol
1% SDS

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use cold TRIzol Reagent if the starting material contains high levels of RNase, such as spleen or pancreas samples.
- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified materials.

- Always use proper microbiological aseptic techniques when working with RNA.

Lyse samples and separate phases

Lyse and homogenize samples in TRIzol Reagent according to your starting material.

- Tissue

Homogenize tissue samples in 1 mL of TRIzol per 50-100 mg of tissue. For small quantities of tissue (1-10 mg), add 800µL of TRIzol. 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 × g for 10 minutes at 4 °C. Transfer the cleared homogenate to a fresh tube.

- Cells grown on monolayer

Lyse cells directly in a culture dish or flask by adding 1 mL of TRIzol per 10cm2 growth area, pipette the cell lysate several times to ensure sufficient cell disruption.

- Cells grown on suspension

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

Protocol for the isolation of RNA

- Add 1ml ice-cold TRIzol solution to 2ml tube containing homogenized sample.
- Vortex 5-10sec and incubate at room temperature for 5-15 min.
- Add 200 µl of Chloroform. Shake tubes vigorously by hand for 15 seconds and incubate them at 4°C for 10 minutes (Do not vortex).
- Centrifuge the samples no more than 12,000× g for 15 min at 2 to 8 °C.
- Transfer the Aqueous phase (upper Phase) to new RNase-free 1.5ml tube; do not disturb the mid phase. Precipitate the RNA by mixing equal volume of Isopropanol.
- Mix by pipetting up and down gently, (Do not vortex) then incubate on ice for 15 min.
- Centrifuge the mixture at 12,000× g at 2 to 8 °C for 15min.
- Discard the supernatant and add 1ml of 75% Ethanol, centrifuge at 4 °C for 5 min, at 8500× g.
- Remove the supernatant and let the pellet to air dried at room temperature for few min (do not over dry the pellet).
- Dissolve pellet in 50µl of DEPC treated water and incubate for 10 min at 55 to 60

Protocol for the isolation of DNA

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.

- DNA Precipitation

Remove any remaining aqueous phase overlying the interphase of the RNA isolation protocol. Add 0.3 mL of 100% ethanol per 1 mL of TRIzol used, and mix samples by inversion. Leave samples at room temperature for 3 minutes, then centrifuge at 2000 × g for 5 minutes at 4 °C.

- DNA Wash

Remove the supernatant to waste or retain for protein isolation. Wash the DNA pellet, with 1 mL of 0.1 M sodium citrate in 10% ethanol per 1 mL of TRIzol used, and mix for 30 minutes at room temperature. Centrifuge samples at 2000 × g for 5 minutes at 4 °C. Two washes are usually sufficient, however for large pellets containing >200 µg of DNA an additional wash may be necessary.

Following the wash steps, add 1.5 mL of 75% ethanol per 1 mL of TRIzol used. Mix for 20 minutes at room temperature, then centrifuge samples at 2000 × g for 5 minutes at 4 °C.

Note: At this stage, samples can be stored for at least one month at 4 °C.

- Re-dissolving the DNA

Air-dry the pellet for 15 minutes. Resuspend the pellet in 8 mM NaOH Remove any insoluble material by centrifugation at 12000 × g for 10 minutes and then transfer the supernatant to another tube.

- Note: Samples dissolved in 8 mM NaOH can be stored overnight at 4 °C. For long-term storage, adjust the pH to 7.5, and adjust the EDTA concentration to 1 mM. Store at -20 °C.

Protocol for the isolation of Protein

- Protein Precipitation

To the retained supernatant (step 2 of the DNA isolation protocol) add 1.5 mL of isopropyl alcohol per 1 mL of TRIzol used. Mix samples for 10 minutes at room temperature the centrifuge at 12000 × g for 10 minutes at 4 °C.

- Protein Wash

Remove supernatant and wash the protein pellet twice. To wash the protein pellet add 2 mL of 0.3 M guanidine hydrochloride in 95% ethanol per 1 mL of TRIzol used. Mix for 20 minutes at room temperature then centrifuge at 7500 × g for 5 minutes at 4 °C.

Note: At this stage, samples can be stored for at least one month at 4 °C, or 12 months at -20 °C.

Following the washing steps, add 2 mL of ethanol and vortex. Mix for 20 minutes at room temperature then centrifuge at 7500 × g for 5 minutes at 4 °C.

- Re-dissolving the Protein

Vacuum dry the protein pellet for 5-10 minutes. Dissolve in 1% SDS by pipetting up and down. For difficult samples incubate at 50 °C. Remove any insoluble material by centrifugation at 10000 × g for 10 minutes at 4 °C and then transfer the supernatant to another tube. Store protein at -20 °C.

Troubleshooting

Observation	Possible cause	Recommended action
A lower yield than expected is observed	The samples were in completely homogenized or lysed.	Decrease the amount of starting material.
		Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in TRIzol Reagent to achieve total lysis.
	The pellet was in completely solubilized	Increase the solubilization rate by pipetting the sample repeatedly, and heat the sample to 50–60°C.
The sample is degraded	Samples were not immediately processed or frozen after collection.	Sample must be processed or frozen immediately after collection.
	Sample preparations were stored at the incorrect temperature.	Store RNA samples at –60 to –70°C. Store DNA and protein samples at –20°C.
The RNA or DNA is contaminated	The interphase/organic phase is pipetted up with the aqueous phase.	Do not attempt to draw off the entire aqueous layer after phase separation.
	The aqueous phase is in completely removed.	Remove remnants of the aqueous phase prior to DNA precipitation.
	The DNA pellet is insufficiently washed with 0.1 M sodium citrate in10% ethanol	Make sure pellet is washed with 0.1 M sodium citrate in 10% ethanol.
The RNA $A_{260/280}$ ratio is low	Sample was homogenized in an insufficient volume of TRIzol Reagent.	Add the appropriate amount of TRIzol Reagent for your sample type.
	The organic phase is in completely removed.	Do not attempt to draw off the entire aqueous layer after phase separation.
The DNA $A_{260/280}$ ratio is low	Phenol was not sufficiently removed from the DNA preparation.	Wash the DNA pellet one additional time in 0.1 M sodium citrate in 10% ethanol.