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Product Information

TRIzol Reagent

Catalog Number	Packaging Size
FP312	100 mL

Storage upon receipt:
- 2-8°C
- Protect from light

Product Description

TRIzol Reagent is a ready-to-use reagent, designed to isolate high quality total RNA (as well as DNA and proteins) from cell and tissue samples, yeast, or bacteria.

TRIzol Reagent allows to perform sequential precipitation of RNA, DNA, and proteins from a single sample. After homogenizing the sample with TRIzol reagent, chloroform is added, and the homogenate is allowed to separate into a clear upper aqueous layer (containing RNA), an interphase, and a blue lower organic layer (containing the DNA and proteins). RNA is precipitated from the aqueous layer with isopropanol. DNA is precipitated from the interphase/organic layer with ethanol. Protein is precipitated from the phenol-ethanol supernatant by isopropanol precipitation. The precipitated RNA, DNA, or protein is washed to remove impurities, and then resuspended for use in downstream applications.

- Isolated RNA can be used in RT-PCR, Northern Blot analysis, Dot Blot hybridization, poly(A)+ selection, in vitro translation, RNase protection assay, and molecular cloning.
- Isolated DNA can be used in PCR, Restriction Enzyme digestion, and Southern Blots.
- Isolated protein can be used for Western Blots, recovery of some enzymatic activity, and some immunoprecipitation.

Required materials not supplied

- Chloroform
- 100% Ethanol
- Isopropanol
- 0.3 M Guanidine hydrochloride in 95% ethanol
- 1% SDS
- microcentrifuge tubes
- Centrifuge capable of reaching 12,000 × g and 4°C

Lyse samples and separate phases

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

- **Tissues:** Add 1 mL of TRIzol Reagent per 10–100 mg of tissue to the sample and homogenize using a homogenizer.
- **Cell grown in monolayer:** Remove growth media; Add 0.3-0.4 mL of TRIzol Reagent per 1 × 10^5-10^7 cells directly to the culture dish to lyse the cells; Pipet the lysate up and down several times to homogenize.
- **Cells grown in suspension:** Pellet the cells by centrifugation and discard the supernatant; Add 0.75 mL of TRIzol Reagent per 0.25 mL of sample (5-10 × 10^6 cells from animal, plant, or yeasty origin or 1 ×10^7 cells of bacterial origin) to the pellet; Pipet the lysate up and down several times to homogenize.

Note: The sample volume should not exceed 10% of the volume of TRIzol Reagent used for lysis.

2. *(Optional)* If samples have a high fat content, centrifuge the lysate for 5 minutes at 12,000 × *g* at 4–10°C, then transfer the clear supernatant to a new tube.

3. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.

4. Add 0.2 mL of chloroform per 1 mL of TRIzol Reagent used for lysis, then securely cap the tube.

5. Incubate for 2–3 minutes.

6. Centrifuge the sample for 15 minutes at 12,000 × *g* at 4°C.

The mixture separates into a lower blue phenol-chloroform, and interphase, and a colorless upper aqueous phase.

7. Discard the aqueous phase containing the RNA, then proceed directly to the next section with the interphase containing the DNA and protein.

Isolate protein

1. Remove any remaining aqueous phase overlying the interphase.

2. Add 0.3 mL of 100% ethanol per 1 mL of TRIzol Reagent used for lysis.

3. Cap the tube, mix by inverting the tube several times.

4. Incubate for 2–3 minutes.

5. Centrifuge for 5 minutes at 2000 × *g* at 4°C to pellet the DNA.

6. Transfer the phenol-ethanol supernatant to a new tube.

7. Add 1.5 mL of isopropanol to the phenol-ethanol supernatant per 1 mL of TRIzol™ Reagent used for lysis.

8. Incubate for 10 minutes.

9. Centrifuge for 10 minutes at 12,000 × *g* at 4°C to pellet the proteins.

10. Discard the supernatant with a micropipettor.

11. Resuspend the pellet in 2 mL of 0.3 M guanidine hydrochloride in 95% ethanol per 1 mL of TRIzol Reagent used for lysis.

12. Incubate for 20 minutes.

Note: The proteins can be stored in wash solution for at least 1 month at 4°C or for at least 1 year at –20°C.

13. Centrifuge for 5 minutes at 7500 × *g* at 4°C.

14. Discard the supernatant with a micropipettor.

15. Repeat step 11–step 15 twice.

16. Add 2 mL of 100% ethanol, then mix by vortexing briefly.

17. Incubate for 20 minutes.

18. Centrifuge for 5 minutes at 7500 × *g* at 4°C.

19. Discard the supernatant with a micropipettor.

20. Air dry the protein pellet for 5–10 minutes.

Note: Do not dry the pellet by vacuum centrifuge.

21. Resuspend the pellet in 200 µL of 1% SDS by pipetting up and down.

Note: To ensure complete resuspension of the pellet, we recommend that you incubate the sample at 50°C in a water bath or heat block.

22. Centrifuge for 10 minutes at 10,000 × *g* at 4°C to remove insoluble materials.

23. Transfer the supernatant to a new tube.

Proceed directly to downstream applications, or store the sample at -20°C.

Trouble shooting

Observation	Observation	Recommended action
A lower yield than expected is observed	The samples were incompletely 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 incompletely 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.