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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
- 75% Ethanol
- 0.1 M sodium citrate in 10% ethanol
- 8 mM NaOH
- HEPES
- microcentrifuge tubes
- Centrifuge capable of reaching 12,000 × g and 4°C

Input sample requirements

Note: Perform DNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at –80°C or in liquid nitrogen until DNA isolation.

Sample type	Sample amount	TRIzol amount
Tissues	<10mg 10-100mg	0.8ml+100µg Glycogen 1 ml
Cells grown in monolayer	3.5-cm culture dish (10 cm^2)	1ml
Cells grown in suspension	10^2-10^5 cells 5-10 x 10^6 cells	0.8ml+100µg Glycogen 1 ml
Blood	100 ul	1 ml

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..

Isolate DNA

1. Precipitate the DNA

a. Remove any remaining aqueous phase overlying the interphase. This is critical for the quality of the isolated DNA.

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

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

d. Incubate for 2–3 minutes.

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

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

The supernatant is used for protein isolation, if needed, and can be stored at –70°C for several months.

2. Wash the DNA

a. Resuspend the pellet in 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 1 mL of TRIzol Reagent used for lysis.

b. Incubate for 30 minutes, mixing occasionally by gentle inversion.

Note: The DNA can be stored in sodium citrate/ethanol for at least 2 hours.

c. Centrifuge for 5 minutes at 2000 × *g* at 4°C.

d. Discard the supernatant with a micropipettor.

e. Repeat step 2a–step 2d once.

Note: Repeat step 2a–step 2d twice for large DNA pellets (>200 µg).

f. Resuspend the pellet in 1.5–2 mL of 75% ethanol per 1 mL of TRIzol Reagent used for lysis.

g. Incubate for 10–20 minutes, mixing occasionally by gentle inversion.

Note: The DNA can be stored in 75% ethanol at several months at 4°C.

h. Centrifuge for 5 minutes at 2000 × *g* at 4°C.

i. Discard the supernatant with a micropipettor.

j. Vacuum or air dry the DNA pellet for 5–10 minutes.

3. Solubilize the DNA

a. Resuspend the pellet in 0.3–0.6 mL of 8 mM NaOH by pipetting up and down.

Note: We recommend resuspending the DNA is a mild base because isolated DNA does not resuspend well in water or Tris buffer.

b. Centrifuge for 10 minutes at 12,000 × *g* at 4°C to remove insoluble materials.

c. Transfer the supernatant to a new tube, then adjust pH as needed with HEPES.

Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C, adjust the pH to 7–8 with HEPES and add 1 mM EDTA.

4. Determine the DNA yield by OD measurement

a. Dilute sample in water or buffer (pH >7.5), then measure absorbance at 260 nm and 280 nm.

b. Calculate the DNA concentration using the formula A260 × dilution × 50 = µg DNA/mL.

c. Calculate the A260/A280 ratio. A ratio of ~1.8 is considered pure.

Typical DNA (A260/280 of 1.6~1.8) yields from various starting materials

Starting material	Quantity	DNA yield
Mammal cells	1 × 10^6 cells	5-7 µg
Fibroblasts	1 × 10^6 cells	5-7 µg
Skeletal muscles and brain	1 mg	2-3 µg
Placenta	1 mg	2-3 µg
Liver	1 mg	3-4 µg
Kidney	1 mg	3-4 µg

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.
The DNA is contaminated	The aqueous phase is incompletely removed.	Remove remnants of the aqueous phase prior to DNA precipitation.
	The DNA pellet is insufficiently washed with 0.1 M sodium citrate in 10% ethanol	Make sure pellet is washed with 0.1 M sodium citrate in 10% ethanol.
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.