9620 Medical Center Drive
Rockville, MD 20850, USA
Web: www.abpbio.com;
www.abpbiotech.com.cn

Product Information

TRIzol RNA isolation reagent

Catalog Number	Packaging Size
FP312	100 mL

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

Product Description

TRIzol RNA isolation 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 RNA isolation reagent** is a monophasic solution of phenol, guanidine isothiocyanate, and other proprietary components which facilitate the isolation of a variety of RNA species of large or small molecular size. **TRIzol RNA isolation reagent** maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization. **TRIzol RNA isolation reagent** allows for simultaneous processing of a large number of samples.

TRIzol RNA isolation 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
- Isopropanol
- 75% Ethanol
- RNase-free water
- microcentrifuge tubes
- Centrifuge capable of reaching 12,000 × g and 4°C

Input sample requirements

Note: Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at –80°C or in liquid nitrogen until RNA 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. Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.

Isolate RNA

1. Precipitate the RNA

a. Add 0.5 mL of isopropanol to the aqueous phase, per 1 mL of TRIzol Reagent used for lysis.

b. Incubate for 10 minutes.

c. Centrifuge for 10 minutes at 12,000 × *g* at 4°C.

Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.

d. Discard the supernatant with a micropipettor.

2. Wash the RNA

a. Resuspend the pellet in 1 mL of 75% ethanol per 1 mL of TRIzol Reagent used for lysis.

Note: The RNA can be stored in 75% ethanol for at least 1 year at -20°C, or at least 1 week at 4°C.

b. Vortex the sample briefly, then centrifuge for 5 minutes at 7500 × *g* at 4°C.

c. Discard the supernatant with a micropipettor.

d. Vacuum or air dry the RNA pellet for 5-10 minutes.

3. Solubilize the RNA

a. Resuspend the pellet in 20–50 µL of RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution by pipetting up and down.

Note: Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.

b. Incubate in a water bath or heat block set at 55-60°C for 10-15 minutes.

4. Determine the RNA yield by OD measurement

a. Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm.

b. Calculate the RNA concentration using the formula A260 × dilution × 40 = µg RNA/mL.

c. Calculate the A260/A280 ratio. A ratio of ~2 is considered pure.

Typical RNA (A260/280 of >1.8) yields from various starting materials

Starting material	Quantity	RNA yield
Epithelial cells	1×10^6 cells	8-15 µg
New tobacco leaf	-	73 µg
Fibroblasts	1×10^6 cells	5-7 µg
Skeletal muscles and brain	1 mg	1-1.5 µg
Placenta	1 mg	1-4 µg
Liver	1 mg	6-10 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 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 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 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 incompletely 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.