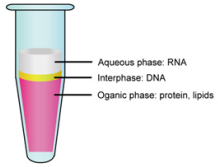
DNA & RNA ISOLATION FROM CELLS WITH TRIzol REAGENT

What is TRIzol?

- TRIzol™ Reagent is a ready-to-use reagent which allows to perform sequential precipitation of RNA, DNA and proteins from a single sample.
- It's 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.
- It maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization.

How does it work?

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 red 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. . The precipitated RNA, DNA, or protein is washed to remove impurities and then resuspended for use in downstream applications.

EQUIPMENT

Fixed Equipment

- Centrifuge
- Heat block at 55–60°C
- Pipettes suitable for measuring 10, 200 and 1000 µl, respectively
- Laminar Flow Hood

Consumables

- Polypropylene microcentrifuge tubes
- Filter pipette tips 10, 200, 1000 µl

REAGENTS

- TRIzol Reagent
- Choloroform

RNA Isolation:

- Isopropanol, 100%
- Ethanol, 75%
- Nuclease-free water

DNA Isolation:

- Ethanol, 100%
- Ethanol, 75%
- 0.1 M sodium citrate in 10% ethanol, pH 8.5
- Nuclease-free water

NB: Nuclease-Free Water is used in various molecular biology applications requiring nuclease-free conditions, such as in processing DNA or RNA. This product is sterile-filtered (0.2 µm), free of RNase and DNase activity, endotoxin-free, and not DEPC-treated.

CAUTION
TRIzol contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant) and may be a health hazard if not handled properly. Always work with TRIzol under the hood, always wear a lab coat and gloves.

IMPORTANT!
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.

1. **LYSE SAMPLES AND SEPARATE PHASES**
   - Add 0.5 mL of TRIzol to the cellular pellet.
   - Pipet the lysate up and down several times to homogenize.
   - Incubate for 5 minutes at room temperature to permit complete dissociation of the nucleoproteins complex.
   - Add 0.1 mL of chloroform, then securely cap the tube.
   - Shake tube vigorously by hand for 15 seconds.
   - Incubate for 2-3 minutes at room temperature.
   - Centrifuge the sample at 12000 x g for 15 minutes at 4°C.
     The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.
   - Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out. The new tube must be marked with the corresponding condition, the initials of the group and specifying "RNA".

     IMPORTANT! Avoid transferring any of the interphase or organic layer into the pipette when removing the aqueous phase.

- Save the interphase and organic phase if you want to isolate DNA.

2. ISOLATE RNA

Always use the appropriate precautions to avoid RNase contamination when preparing and handling RNA.

2.1 Precipitate the RNA

- Add 0.250 mL of 100% isopropanol to the aqueous phase.
- Incubate at room temperature for 10 minutes.
- Centrifuge for 10 minutes at 12000 × g at 4°C.
  Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.
- Discard the supernatant with a pipette.

2.2 Wash the RNA

- Wash the pellet with 0.5 mL of 75% ethanol.
- Vortex the sample briefly then centrifuge for 5 minutes at 7500 × g at 4°C.
- Discard the supernatant.
- Vacuum or air dry the RNA pellet for 5–10 minutes.

IMPORTANT! Do not dry the pellet by vacuum centrifuge. Do not let the RNA pellet dry, to ensure total solubilization of the RNA. Partially dissolved RNA samples have an A230/280 ratio <1.6.

2.3 Solubilize the RNA

- Resuspend the pellet in 25 µL of Nuclease-free water by pipetting up and down.
- Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.

Proceed to downstream applications, or store the RNA at –80°C.

2.4 Determine the RNA yield

Method	Procedure
Absorbance Absorbance at 260 nm provides total nucleic acid content, while absorbance at 280 nm determines sample purity. Since free nucleotides, RNA, ssDNA, and dsDNS absorb at 260 nm, they all contribute to the total absorbance of the sample.	1. Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm. 2. Calculate the RNA concentration using the formula A260 × dilution × 40 = µg RNA/mL. 3. Calculate the A260/A280 ratio. A ratio of ~2 is considered pure. RNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectophotometer. Refer to the instrument's instructions for more information.

3. ISOLATE DNA

Isolate DNA from the interphase and the lower phenol-chloroform phase saved from "Lyse samples and separate phases"

3.1 Precipitate the DNA

- Remove any remaining aqueous phase overlying the interphase.
  This is critical for the quality of the isolated DNA.
- Add 0.150 mL of 100% ethanol.
- Cap the tube, mix by inverting the tube several times.
- Incubate for 2–3 minutes.
- Centrifuge for 5 minutes at 2000 × g at 4°C to pellet the DNA.
- Remove the phenol-ethanol supernatant and save it in a new tube.

3.2 Wash the DNA

- Wash the DNA pellet with 0.5 mL of sodium citrate/ethanol solution (0.1 M sodium citrate in 10% ethanol, pH 8.5).
- Incubate for 30 minutes, mixing occasionally by gentle inversion.
- Centrifuge for 5 minutes at 2000 × g at 4°C.
- Discard the supernatant.
- Repeat wash, once.
- Resuspend the pellet in 1 mL of 75% ethanol.
- Incubate for 10–20 minutes, mixing occasionally by gentle inversion.
- Centrifuge for 5 minutes at 2000 × g at 4°C.
- Discard the supernatant.
- Vacuum or air dry the DNA pellet for 5–10 minutes.

3.3 Solubilize the DNA

- Resuspend the pellet in 30 µL of Nuclease-free water by pipetting up and down.

Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C

3.4 Determine the DNA yield

Method	Procedure
Absorbance Absorbance at 260 nm provides total nucleic acid content, while absorbance at 280 nm determines sample purity. Since free nucleotides, RNA, ssDNA, and dsDNS absorb at 260 nm, they all contribute to the total absorbance of the sample.	1. Dilute sample in water or buffer (pH >7.5), then measure absorbance at 260 nm and 280 nm. 2. Calculate the DNA concentration using the formula A260 × dilution × 50 = µg DNA/mL. 3. Calculate the A260/A280 ratio. A ratio of ~1.8 is considered pure. DNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectophotometer. Refer to the instrument's instructions for more information.