TRIZOL-BASED RNA AND DNA ISOLATION

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Working Solutions

Reagents and Solutions	**Storage**
0.1M Sodium Citrate	4℃
1kb Gel Ladder	4℃ or RT
1M Tris-HCl (T5085, TEKNOVA)	Acids cabinet, RT
6X Orange Loading Dye (B7022S, New England Biolabs)	RT
70% Ethanol in Nuclease-Free Water	-20 ℃
75% Ethanol	-20 ℃
8mM NaOH	Bases cabinet, RT
Agarose (S-26942, Thermo Scientific)	RT
Chloroform (4440-04, Macron™)	Flammable cabinet, RT
Ethanol (E7023, Sigma-Aldrich)	Flammable cabinet, RT
GelRed (SCT123, Millipore)	4℃
GlycoBlue™ (AM9515, Invitrogen)	-20℃
Isopropanol (190764, Sigma-Aldrich)	Flammable cabinet RT (~20-22℃)
NaOH (S318-3, Thermo Scientific)	Bases cabinet, RT
Nuclease-free water	RT
10X TBE Buffer	RT

**RT: room temperature (~20-22℃)*

Sample Collection

Samples can be collected in microtubes and fast-frozen in liquid nitrogen.
Alternatively, samples can be collected directly into Trizol reagent (recommended: 100µl-250µl, step 2 note below). Keep on ice while collecting.
Note: The volume of the tissue should not exceed 10% of the volume of the Trizol Reagent.

** Store at -80℃ if not processed immediately after. **

RNA Extraction

This protocol has been modified from the manufacturer instructions: TRIzol Reagent User Guide (Pub.No. MAN0001271 D).

Use filter tips throughout
1. Carry out the Trizol reagent and chloroform steps under the hood! Make sure to discard these into relevant waste bottles.

2. Homogenize tissue samples in **1 mL of Trizol Reagent*** using a clean and autoclaved pestle in a low adhesion 1.5mL microtube. This is probably the most important part: tissue must be completely homogenized. **Recommended amount of TRIzol: 1 ml per 50–100 mg of tissue)*

Note: It is best to not have the full Trizol amount (500-1000µL) in the tube during homogenization so it doesn't overflow with pestle. So put less in at first, homogenize and then add to equal necessary final volume. Do not vortex!

3. Keep on ice for **5 min**.

4. Add **200 µL chloroform** (1:5, chloroform:TRIzol). Do not vortex, mix by flicking. Invert tubes - want a milky pink solution. Keep on ice for **5 min**.

5. Centrifuge for **5 min at 4°C** (using the centrifuge in the Rowe third floor cold room) at **12,000 *g*** (rcf). If the phases aren't separated, invert tubes more completely and recentrifuge.

6. Transfer aqueous phase (clear part being very careful to not get white slip of DNA between clear and pink parts) to new low-adhesion tubes. Use the p100 pipette and then p10 very carefully.

7. Add 1uL of glycoblue coprecipitant to the aqueous phase that has been collected into the new low-adhesion tubes.

8. Add **500uL of isopropanol***. Mix by flicking. **Isopropanol ratio should be 1:2, 500µL isopropanol per 1mL of TRIzol.*

9. Incubate at **-20°C for 20 min.**

10. Centrifuge for **10 min at 12,000 x g at 4°C.**

Note: Every time centrifuging, it is helpful to put the hinge of the tube towards the outside of the centrifuge so you eventually know where the RNA pellet should be found.

11. RNA should appear as a white (blue if you added the glycoblue) pellet, discard supernatant.

12. Add **1 mL 70% EtOH** (that has been prepared with nuclease-free water and has been kept in the -20). Mix by flicking.

*Note: Now would be a good time to turn the heat block or water bath on and set to **55-60°C**.*

13. Spin for **5 min at 4°C at 12,000 x g.**

14. Remove EtOH carefully (First carefully and slowly with a p100 and then with a p10)

15. Dry pellet for 5 min by opening the tube (doesn't need to be on ice) and letting sit. Repeat as needed but if one dries faster than others, put that one on ice. Typically takes ~15-20 minutes under the hood, checking every 5 minutes.
15.1. Do not let the RNA pellet dry completely, as this will greatly decrease its solubility.
15.2. A more complete evaporation of ethanol is required when RNA samples are to be used in RT-PCR.

16. Resuspend in 15 uL nuclease-free water. Flick to mix.

17. Incubate in a **55-60C°Cwater bath or heat block for 5-10 min**.

18. RNA can be used immediately for cDNA synthesis, further purified for sequencing applications, or stored at **−70 °C to −80 °C** until use.

Measuring Concentration of RNA:
Flick, spin down, and measure concentration. Use 1µL of the RNA template to measure concentration with NanoDrop.

Checking mRNA on Agarose Gel:

1. Make 1% agarose GelRed (SCT123, Millipore) gel:
    1.1. Measure out TBE buffer into flask and measure out agarose. Put agarose into a flask with a TBE buffer and microwave: 65 seconds for small gels, 2 minutes 30 seconds for large gels. Add GelRed and swirl.
    1.2. For small gel: 0.6g agarose + 60mL TBE buffer + 6uL Gel Red
    1.3. For large gel: 1.4g agarose + 140mL TBE buffer + 14uL Gel Red

2. Put together gel mold with comb during microwaving. Many of our large gel molds leak and you will need to line each side with parafilm to stop that.
3. Pour agarose mixture into mold with a comb and let it solidify.
4. Run on 1% agarose gel to check quality for ~10/15 minutes. Image gel and run longer if needed. BioRad Gel Scanner is next to the Nanodrop in the Rowe fourth floor equipment room.
5. Use 1 ul of RNA and dilute to 10 uL volume RNA + loading dye
   - 1 ul RNA
   - 2 ul 6X loading dye
   - 9 ul nuclease-free water
6. If RNA concentration was very low, increase the volume of RNA and add nuclease water up to 10uL.

RNA sequencing

If RNA samples are meant for RNA-sequancing, we recommend purification with QIAGEN RNeasy Mini Kit including step of DNase I treatment. Check RNA quality with Bioanalyzer or Tape Station.

DNA extraction

This protocol has been slightly modified from the manufacturer instructions: https://documents.thermofisher.com/TFS-Assets/LSG/manuals/MAN0016385_TRIzol_Reagent_DNA_Isol_UG.pdf

1. Precipitate the DNA
   - 1.1. Remove any remaining aqueous phase overlying the interphase. This is critical for the quality of the isolated DNA.
   - 1.2. Add **0.3 mL of 100% EtOH** per 1 mL of TRIzol™ Reagent used for lysis. Cap the tube, mix by inverting the tube several times.
   - 1.3. Incubate for **2–3 min**.
   - 1.4. Centrifuge for **5 min at 2000 × g at 4°C** to pellet the DNA.
   - 1.5. Transfer the phenol-ethanol supernatant to a new tube. The supernatant is used for protein isolation [refer to TRIzol™ Reagent User Guide (Pub. No. MAN0001271)], if needed, and can be stored at –70°C to 80°C for several months.

2. Wash the DNA
   - 2.1. 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.

2.2. Incubate for 30 minutes, mixing occasionally by gentle inversion. Note: The DNA can be stored in sodium citrate/ethanol for at least 2 hours.
2.3. Centrifuge for 5 minutes at 2000 × g at 4°C.
2.4. Discard the supernatant with a micropipette.
2.5. Repeat step 2a–step 2d once. *Note: Repeat step 2a–step 2d twice for large DNA pellets (>200 µg). Resuspend the pellet in 1.5–2 mL of 75% ethanol per 1 mL of TRIzol™ Reagent used for lysis.*
2.6. Incubate for 10–20 minutes, mixing occasionally by gentle inversion. *Note: The DNA can be stored in 75% ethanol for several months at 4°C.*
2.7. Centrifuge for 5 minutes at 2000 × g at 4°C.
2.8. Discard the supernatant with a micropipette. Vacuum or air dry the DNA pellet for 5–10 minutes. IMPORTANT! Do not dry the pellet by vacuum centrifuge.

3.Solubilize the DNA

3.1. 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.
3.2. Centrifuge for 10 minutes at 12,000 × g at 4°C to remove insoluble materials.
3.3. Transfer the supernatant to a new tube, then adjust pH as needed with HEPES or Tris-HCL (see above **DNA Neutralization**).
3.4. 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

DNA Neutralization

DNA is dissolved in **8 mM NaOH** and must be neutralized before storage or downstream applications.

Neutralization reagent: 1 M Tris-HCl, pH 7.5

Procedures

1. Note the volume of DNA dissolved in 8 mM NaOH.
2. Add **1 µL of 1 M Tris-HCl (pH 7.5) per 100 µL of DNA solution**.
3. Mix gently by pipetting or brief vortexing.
4. Spin down briefly and incubate at room temperature for **5–10 minutes** to allow pH equilibration.

Volume guide

1. 50 µL DNA → add **0.5 µL** Tris-HCl
2. 100 µL DNA → add **1.0 µL** Tris-HCl
3. 200 µL DNA → add **2.0 µL** Tris-HCl

This brings the solution to approximately **pH 7.5–8.0**, suitable for storage or enzymatic reactions.

Notes

1. For long-term storage, DNA can be transferred to the TE buffer **(10 mM Tris, 1 mM EDTA, pH 8.0)** after neutralization.
2. If precise pH confirmation is required, test a parallel NaOH-only control diluted 1:10 using pH paper or micro-pH strips.

Preparation of Working Solutions

70% Ethanol (v/v, RNase-free, pre-chilled)

Use nuclease-free water. Store at −20 °C in clearly labeled 15mL Falcon tubes.

1. To make 10 mL: 7 mL 100% ethanol + 3 mL nuclease-free water.

Notes: Mix well, aliquot if helpful, and pre-chill before use.

0.1 M Sodium Citrate in 10% Ethanol, pH 8.5

Best practice: adjust pH before adding ethanol.

To make 100 mL:

1. Dissolve **2.94 g** trisodium citrate dihydrate (MW ≈ 294.1) in ~80 mL nuclease-free water.
2. Adjust **pH to 8.5** with HCl or NaOH as needed.
3. Bring volume to **90 mL** with water.
4. Add **10 mL** 100% ethanol (final **10% v/v**).
5. Mix; store at **4 °C** (label pH and date).

75% Ethanol (v/v)

1. **10 mL:** 7.5 mL 100% ethanol + 2.5 mL water
   Store at **4 °C** (for DNA wash) or **−20 °C** if you prefer consistent temp.

8 mM NaOH (DNA solubilization)

Prepare fresh or keep a small bottle tightly capped.

1. **From pellets:**

   a. **100 mL:** dissolve **0.032 g** NaOH in water, bring to 100 mL
   b. **250mL:** dissolve **0.08 g** NaOH in water, bring to 250 mL

Store at **RT**; keep closed (CO_2 absorption will drift pH).

HEPES

Keep **1 M HEPES, pH 7.5–8.0** on hand (commercial or lab-made).

1. **To prepare 1 M HEPES, pH 7.5 (100 mL):** dissolve **23.83 g** HEPES free acid in ~80 mL water, adjust to pH 7.5 with NaOH, bring to 100 mL. Filter-sterilize; store at **RT** or **4 °C**.

Use dropwise to titrate DNA solution to **pH 7–8** before long-term storage (add **1 mM EDTA** if desired for chelation).

DNA Storage Buffer (TE Buffer)

If you plan to keep DNA for months to years, prepare a **TE buffer** using your Tris-HCl stock:

100 mL of 1× TE, pH 8.0:

1. 1 mL 1 M Tris-HCl (pH 8.0) → 10 mM final
2. 0.2 mL 0.5 M EDTA → 1 mM final
3. Add nuclease-free water to 100 mL total volume

Filter-sterilize, label, and store at **room temperature or 4 °C**.

Use: DNA stable for years at –20 °C in 1× TE buffer.

1 M Tris-HCl, pH 7.5–8.0

Reagents

1. Tris base (Tris[hydroxymethyl]aminomethane)
2. Concentrated HCl (≈12 M)
3. Nuclease-free water

100 mL of 1 M Tris-HCl

1. Weigh **12.11 g Tris base** and transfer to a clean 250 mL beaker or flask.
2. Add ~80 mL nuclease-free water and stir to dissolve completely.
3. Check pH with a pH meter.
    a. Slowly add concentrated **HCl** drop by drop until **pH = 7.5–8.0** (typical for DNA neutralization or storage).
    b. For pH 7.5 → slightly more acidic (good for neutralizing DNA in NaOH).
    c. For pH 8.0 → slightly more basic (good for storage buffer).
4. Bring the volume up to **100 mL** with nuclease-free water.
5. Filter-sterilize through a **0.22 µm filter** if long-term storage is planned.
6. Label the bottle: "*1 M Tris-HCl pH 7.5 (RNase/DNase-free), date, initials, and store at room temperature or 4°C*".