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Protocol status: Working

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🌐 Total RNA extraction

📖 PLOS One

Yuan Yao¹

¹Inner Mongolia People`s Hospital

Yuan Yao

ATTACHMENTS

[Trizol_Plus_man.pdf](#)

MATERIALS

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🔗 DNase I (RNase-free) - 1,000 units **New England Biolabs Catalog #M0303S**

🔗 RNase-free Water **Contributed by users**

🔗 RNaseZap® **Thermo Scientific Catalog #AM9780**

- 1 Remove growth media.
- 2 Add 1 mL of TRIzol™ Reagent per 1×10^5 – 10^7 cells directly to the culture dish to lyse the cells.
- 3 Pipet the lysate up and down several times to homogenize.
- 4 Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
- 5 Add 0.2 mL of chloroform or 50 μ L of 4-bromoanisole per 1 mL of TRIzol™ Reagent used for lysis, then securely cap the tube.
- 6 Incubate for 2–3 minutes.
- 7 Centrifuge the sample for 15 minutes at $12,000 \times g$ at 4°C.
- 8 Transfer ~600 μ L of the colorless, upper aqueous phase containing the RNA to a new tube.

- 9 Add an equal volume of 70% ethanol, then mix well by vortexing.
- 10 Invert the tube to disperse any visible precipitate that may form after adding ethanol.
- 11 Transfer up to 700 μ L of the sample to a spin cartridge (with collection tube).
- 12 Centrifuge at 12,000 $\times$ g for 15 seconds.
- 13 Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
- 14 Repeat step 2a–step 2c until the entire sample has been processed.
- 15 Add 700 μ L of Wash Buffer I to the spin cartridge.
- 16 Centrifuge at 12,000 $\times$ g for 15 seconds.

- 17** Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
- 18** Add 500 μL of Wash Buffer II to the spin cartridge.
- 19** Centrifuge at $12,000 \times g$ for 15 seconds.
- 20** Discard the flow-through, then reinsert the spin cartridge into the same collection tube.
- 21** Repeat step 18-step 20 once.
- 22** Centrifuge at $12,000 \times g$ for 1 minute to dry the membrane.
- 23** Discard the collection tube, then insert the spin cartridge into a recovery tube.
- 24** Add 30 μL -3 $\times$ 100 μL (3 sequential elutions with 100 μL each) of RNase-free water to the center of the spin cartridge.
- 25** Incubate for 1 minute.

- 26** Centrifuge at $>12,000 \times g$ for 2 minutes.
- 27** Discard the spin cartridge.
- 28** RNA integrity was verified by electrophoresis on a 1.2% agarose gel containing formaldehyde, and post stained with 1.0 $\mu\text{g/ml}$ ethidium bromide.
- 29** Determine the quality and quantity of the purified RNA using UV absorbance at 260 nm.