Jun 06, 2025

RNA Extraction from Nematodes Using Trizol

DOI
dx.doi.org/10.17504/protocols.io.kqdg3wje7v25/v1

Manuela R. Kieninger[1]

[1]Blaxter Faculty, Wellcome Sanger

Manuela R. Kieninger
Blaxter Faculty, Wellcome Sanger

DOI: https://dx.doi.org/10.17504/protocols.io.kqdg3wje7v25/v1

Protocol Citation: Manuela R. Kieninger 2025. RNA Extraction from Nematodes Using Trizol. **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg3wje7v25/v1**

Protocol status: Working
We use this protocol and it's working

Created: June 03, 2025

Last Modified: June 06, 2025

Protocol Integer ID: 219468

Keywords: nematode, C. elegans, RNA, RNA extraction, Trizol, Sequencing, total RNA, rna extraction from nematode, whole rna extraction from nematode bulk sample, rna extraction, whole rna extraction, nematode bulk sample, using trizol, trizol this protocol, rna, nematode, several micrograms of rna, remaining dna, dnase, dna, rin value

Funders Acknowledgements:
Wellcome Sanger
Grant ID: 220540/Z/20/A

Abstract

This protocol describes whole RNA extraction from nematode bulk samples using Trizol.
The RNA is then treated with DNase to remove any remaining DNA.
Quality control usually shows a RIN value of 8 or higher.
Depending on the input you get several micrograms of RNA.

Materials

- Trizol e.g. TRIzol ®Reagent Ambion by life technologies Cat#: Invitrogen™ 10296010
- RNase free 1.5 ml tubes Safe-lock
- RNase free 2.0 ml tubes Safe-lock
- 4 °C cooled centrifuge for 1.5 ml and 2.0 ml tubes e.g. Eppendorf Centrifuge 5425 R
- Isopropanol
- 3M Na Acetate
- 75% Ethanol
- Chloroform-isoamyl alcohol mixture 49:1 e.g. Sigma Aldrich 25668-100ML
- RNA elution buffer e.g. MagMAX™ Total RNA Elution Buffer Cat#: A41043
- Tips (20 µl, 200 µl, 1000µl) and pipette set
- wet ice
- Turbo DNA-free Kit: TURBO DNA-*free*™ Kit, Invitrogen ™ Cat#: AM1907
- Eppendorf ThermoMixer® C Catalog No. 5382000031
- Eppendorf SmartBlock 1.5 mL Catalog No. 5360000038
- Chemical hood
- Liquid nitrogen
- Long tweezers (about 30 cm)
- Dewar for liquid nitrogen
- RNase*Zap*™ RNase Decontamination Solution Invitrogen ™ Cat#: AM9780

For quality control:

- NanoDrop™ One, Thermo Scientific™, Cat#: ND-ONE-W
- Qubit e.g. Qubit™ 4 Fluorometer, with WiFi, Invitrogen™, Cat#: Q33238
- Qubit™ Assay Tubes, Invitrogen ™ Cat#: Q32856
- Qubit™ RNA Broad Range (BR)Assay Kit, Invitrogen™ Cat#: Q10210
- Tapestation Instrument e.g. 4150 TapeStation System Part Number: G2992AA, Agilent Technologies
- Optical tube strip caps (8x strip), Part Number: 401425, Agilent Technologies
- Optical tube strips (8x Strip), Part Number: 401428, Agilent Technologies
- RNA Tape, RNA ScreenTape Analysis Part Number:5067-5576, Agilent Technologies
- RNA ladder, RNA ScreenTape Analysis Part Number:5067-5578, Agilent Technologies
- RNA ScreenTape Sample Buffer, Part Number 5067-5577, Agilent Technologies

Troubleshooting

Safety warnings

- This protocol requires you to wear a lab coat and Phenol- and Chloroform-safe gloves!
- Work under a chemical safety cabinet while working with Trizol and Chloroform!
- Please collect the waste in a suitable container and dispose of the waste in accordance with your local regulations.
- Make sure to wear appropriate PPE for handling liquid nitrogen!

Before start

The extraction can be started with a frozen sample (from -80°C freezer) or as fresh sample (bulk harvest).
I get plenty of high-quality RNA from a worm pellet of 20 mg. Higher input is of course possible.

Snap freeze cycles in Trizol

30m

1 Take fresh nematodes in an **2.0 ml safe-lock tube** spun down in a centrifuge and remove as much supernatant as possible. Or take a previously frozen pellet of nematodes stored in a ultra-low temperature freezer. Keep on ice until Trizol is added.

1.1 If the nematodes are not in a 2.0 ml **safe-lock** tube move them to a 2.0 ml **safe-lock** tube.

2 Add 1 ml Trizol to the sample.

3 Vortex the tube for 10 sec.

4 Lower the tube in liquid nitrogen with long tweezers and wait for 30 sec.

5 Take the tube out with long tweezers and let them thaw.

5.1 For thawing you can use a Eppendorf Thermomix C set to 37 °C and 1000 rpm.

6 As soon as the sample with Trizol is thawed vortex the sample and freeze again in liquid nitrogen.

7 Repeat the freezing thawing cycles for three cycles in total.

8 After the last thaw vortex the sample for 30 sec and do the next steps in a chemical hood.

Trizol extraction of RNA

1h 10m

9 Incubate the sample for 5 min in room temperature.

5m

10 Add 200 µl Chloroform with Isoamylalcohol and vortex for 1 min. 2m

11 Incubate at room temperature for 3 min. 3m

12 Centrifuge the sample @ 4 °C for 15 min @ 14.000 rcf. 15m

13 Transfer the upper aqueous phase to a new 1.5 ml tube. Avoid taking the interphase.

14 Add 0.1 x of the total volume 3M Na Acetate and mix. Then add 0.7 x of the volume Isopropanol and invert at least 20 times.

15 Incubate on ice for 10 min. 10m

16 Centrifuge the sample @ 4 °C 14.000 rcf for 10 min. 10m

17 You should see a white pellet at the 1.5 ml tube. Discard the supernatant without disturbing the pellet.

18 Add 1 ml 75% Ethanol (cold) and short vortex for 3 sec.

19 Centrifuge for 5min @ 8000 rcf 4 °C. 5m

20 Repeat the Ethanol wash. 5m

21 Remove all the Ethanol. Then short spin and pipette off the last remaining Ethanol.

22 Let the pellet air dry for up to 5 min. For small pellets I dry only 2 min.

5m

23 Add 200 µl elution buffer. You can also use less if your pellet is small. I would not recommend using less than 100 µl.

24 If the pellet does not dissolve, you can incubate the sample for 10 min @ 37 °C. Pipette mix the sample and proceed to DNAse treatment. 10m

DNase treatment 27m

25 I use the Invitrogen TURBO DNA-free Kit.

26 Add 0.1x of the elution volume 10x TURBO DNase Buffer and pipette mix.

27 Add 1 µl TURBO DNase Enzyme.

28 Incubate @ 37 °C for 20 min (max. 30 min). 20m

29 Re-suspend the Inactivation Reagent by vortexing
(if the liquid is difficult to pipette, add 25% of the bead volume Nuclease-free water and vortex again).

30 Add 0.1 x of the volume (1µl/10µl) Inactivation Reagent
but min. 2 µl.

31 Pipette mix and incubate 5 min @ room temperature and flick the tubes for mixing 2 to 3 times during incubation. 5m

32 Centrifuge 10.000 rcf for 1:30 min @ room temperature. 2m

33 Take off supernatant with RNA to a new cold 1.5 ml tube and put the sample on ice.

Quality Control for RNA and storage

40m

34 Measure 1 µl with the Nanodrop. Record the values.

5m

35 Measure 1 µl with the RNA Broad Range Qubit Kit. Record the concentration in ng/µl.

5m

36 Measure the RIN value of the extracted RNA by using 1 µl with the Agilent Tapestation and the RNA tape kit.

30m

37 The RNA should be stored in a ultra-low temperature freezer set to minus 70 to minus 80 °C.