

C. elegans Total RNA isolation in microcentrifuge tubes

Materials

- Samples: live *C. elegans*.
- Bead lysis kit: 1.5 mL RED RINO/Eppendorf Lysis Kit.
- RNA isolation kit: magNEAT Tissue RNA Extraction Kit (Kit ID: 2TRMN-50) from Microzone^μ.

Method: Homogenization

1. Add 450 μ L Lysis Buffer and 20 μ L proteinase K into each lysis kit tube.
2. For each tube, obtain a 500 μ L suspension of *C. elegans* in PBS solution.
3. Centrifuge the suspension for 2 minutes at 9,000 x g to pellet the *C. elegans*.
Note: Pellet sizes may range from 10 – 20 μ L.
4. Remove and discard the supernatant.
5. Resuspend the pellet with 50 μ L lysis buffer and then transfer into the lysis kit tube.
6. Set the Bullet Blender at speed 12, time 3 minutes and homogenize the samples. If using other homogenizer models, refer to the manufacturer's instructions for appropriate settings.
7. Centrifuge for 2 minutes at 9,000 x g to remove the foam.
8. Remove the tubes and visually inspect the samples to confirm complete homogenization.

Method: Extraction

Nucleic Acid Binding:

1. Prepare new microtubes containing 200 μ L PBS.
2. Transfer 320 μ L of the homogenate to the new tubes containing PBS.
3. Centrifuge tubes at 13,000 x g for 5 minutes.
4. Transfer 450 μ L of supernatant into new microfuge tubes, being careful not to disturb the pellets or transfer any debris. Place the tubes on the tube rack.
5. Add 450 μ L Binding Buffer to the supernatant in each tube and mix thoroughly by inversion.
6. Vortex the magNEAT beads thoroughly and add 30 μ L to each sample tube. Mix by inversion until the sample is homogenous. Allow to stand for 5 minutes, mixing again halfway through.
7. Transfer the tube rack to the magnetic stand and allow the magnetic beads to separate.
8. Discard the supernatant and remove the tube rack from the magnetic stand.

Washing I:

9. Add 700 μ L Wash Buffer 1, mix thoroughly by inversion, ensuring all beads have been detached from the tube walls.
10. Place the tube rack onto the magnetic stand and allow the magnetic beads to separate.

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11. Discard the supernatant then remove the tube rack from the magnetic stand. Do not disturb the magnetic beads.
12. Add 700 μ L Wash Buffer 2, mix thoroughly by inversion to completely resuspend the beads.
13. Transfer the tube rack to the magnetic stand and allow the magnetic beads to separate.
14. Discard the supernatant and remove the tube rack from the magnetic stand.
Note: *Be careful not to disturb the magnetic beads.*
15. Repeat steps 12-14 for a total of 2 washes with Wash Buffer 2.
16. Centrifuge samples at 2,000 x g for 3 minutes.
17. Use a 20 μ L pipette to remove any remaining wash buffer from the tubes. Do not disturb the magnetic beads.
18. Leave caps open to air dry the magnetic beads at room temperature for 5 minutes.
Note: *Do not over dry the magnetic beads as this can cause RNA degradation.*

Elution and DNase I Treatment:

19. Add 100 μ L of RNA Elution Buffer to each tube. Mix by tapping to ensure that the magnetic beads are completely resuspended.
20. Prepare the DNase I mastermix. Each sample requires 100 μ L of DNase I reaction buffer and 4 μ L of DNase I. Mix by pipetting up and down. Do NOT vortex.
21. Add 104 μ L of DNase I mastermix to each sample and mix by pipetting up and down to fully resuspend the magnetic beads.
Note: *Do NOT vortex samples containing DNase I.*
22. Incubate samples at room temperature for 10 minutes.

Washing II:

23. Add 200 μ L of RNA Binding Buffer to each tube and mix well by inversion.
24. Add 400 μ L of Wash Buffer 2, mix well by inversion.
25. Place the tube rack onto the magnetic stand and allow the magnetic beads to separate.
26. Discard the supernatant then remove the tube rack from the magnetic stand.
Note: *Be careful not to disturb the magnetic beads.*
27. Add 400 μ L of Wash Buffer 2 and mix well by inversion.
28. Place tube rack onto the magnetic stand and allow the magnetic beads to separate.
29. Discard the supernatant then remove the tube rack from the magnetic stand.
Note: *Be careful not to disturb the magnetic beads.*
30. Centrifuge samples at 2,000 x g for 3 minutes.
31. Use a 20 μ L pipette to remove any remaining wash buffer from the tubes. Do not disturb the magnetic beads.
32. Leave caps open to air dry the magnetic beads at room temperature for 5 minutes.
Note: *Do not over dry the magnetic beads as this can cause RNA degradation.*

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Elution II:

33. Add 100 μL of RNA Elution Buffer to each tube. Mix by tapping to ensure that the magnetic beads are completely resuspended.
34. Place the tube rack back onto the magnetic stand and allow the magnetic beads to separate.
35. Transfer the clear supernatant containing the RNA into new microfuge tubes for downstream processing. Be careful not to disturb the magnetic beads.
36. Analyze RNA quality ($\text{OD}_{260}/\text{OD}_{280}$) and yield using NanoDrop and gel (as shown in Figure 1 and Table 1).
37. Isolated RNA can be stored at -80°C .

Figure 1. In-house data using Bioanalyzer.

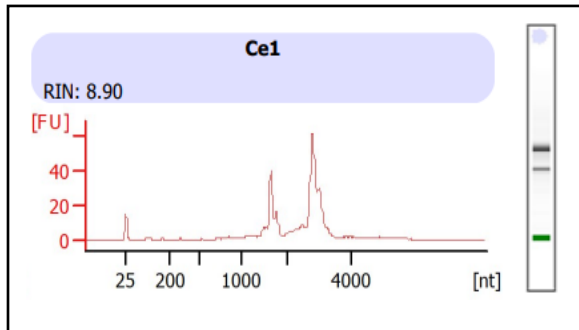


Table 1. In-house data using NanoDrop.

Tissue	Yield: $\mu\text{g}/10\mu\text{L}$ Pellet	$\text{OD}_{260/280}$
C. elegans 1	1.618	1.99
C. elegans 2	1.874	1.92