Drosophila RNA isolation in microcentrifuge tubes

Materials

- Samples: 50 mg of frozen or fresh drosophila.
- Bead lysis kit: 1.5 mL NAVY RINO/Eppendorf Lysis Kit or 2 mL tube filled with beads.
- RNA isolation kit: magNEAT Tissue RNA Extraction Kit (Kit ID: 2TRMN-50) from Microzone $^{\mu}$.

Method: Homogenization

1. Add 500 µL Lysis Buffer and 20 µL proteinase K into each lysis kit tube.
2. Weigh up to 50 mg of tissue samples and transfer to the buffer-filled tubes.
3. 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.
4. Remove the tubes and visually inspect the samples to confirm complete homogenization.

Method: Extraction

Nucleic Acid Binding:

1. Prepare new microtubes containing 200 µL PBS.
2. Transfer 320 µL of the homogenate to the new tubes containing PBS.
3. Centrifuge tubes at 13,000 x g for 5 minutes.
4. Transfer 450 µL 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 µL Binding Buffer to the supernatant in each tube and mix thoroughly by inversion.
6. Add 30 µL of magNEAT magnetic beads to each sample tube. Mix thoroughly by inversion until the sample is homogenous. Allow to stand for 5 minutes, mixing again halfway through.
   Note: *Vortex magNEAT beads thoroughly before adding to ensure they are resuspended.*
7. Transfer the tube rack to the magnetic stand and allow to sit for 1-2 minutes, until the magnetic beads are separated.
8. Discard the supernatant and remove the tube rack from the magnetic stand.

Washing I:

9. Add 700 µL 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.
11. Discard the supernatant then remove the tube rack from the magnetic stand.
    Note: *Be careful not to disturb the magnetic beads.*
12. Add 700 µL 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.

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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 µL 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 µL 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 µL of DNase I reaction buffer and 4 µL of DNase I. Mix by pipetting up and down. Do NOT vortex.
21. Add 104 µL 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 µL of RNA Binding Buffer to each tube. Mix well by inversion, ensuring all beads are resuspended.
24. Add 400 µL 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 µL of Wash Buffer 2 and mix well by inversion. Ensure beads are detached from the tube walls.
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 µL 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 (OD_{260}/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.

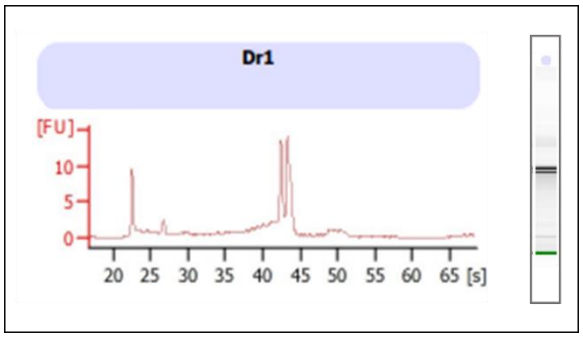

Table 1. In-house data using NanoDrop.

Tissue	Yield: µg/10µL Pellet	$OD_{260/280}$
Drosophila 1	1.432	1.61
Drosophila 2	1.238	1.52