Xenopus RNA Extraction in Microcentrifuge Tubes

RS18-0245A.XEN

Materials

- Navy or PC2 Bead Lysis Kit
- MAGneat Tissue RNA Extraction Kit

Sample

- Xenopus

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Supplied in the Kit:

MAGneat Tissue RNA Lysis Kit Contents	
Lysis Buffer	27.5 mL
Binding Buffer	10.5 mL
MAGneat Magnetic Beads	1.65 mL
Wash Buffer 1	15 mL
Proteinase K	22 mg
DNase I	2.75 mg
DNase I Reaction Buffer	220 µL
RNA Elution Buffer	11 mL

To Be Supplied by User:

- Phosphate-Buffered Saline
- 100% Ethanol
- 100% Isopropyl Alcohol
- Molecular Grade Water
- Magnetic Tube Rack, e.g. Magnetic Rack Assembly (MAGRACK8) shown in Appendix A.

Methods

Buffer Preparation

✓ Lysis Buffer: Ready to use.

___ Binding Buffer: Add 24.5 mL isopropyl alcohol to the RNA Binding Buffer bottle.

___ Wash Buffer 1: Add 22.5 mL of 100% ethanol to the Wash Buffer 1 bottle.

___ Wash Buffer 2 (75% ethanol): 3 parts of 100% ethanol to 1-part of molecular grade water, prepare fresh each day, producing 2.3 mL per sample with 10% excess.

✓ DNase Reaction Buffer: Ready to use.

✓ RNA Elution Buffer: Ready to use.

Enzyme Reconstitution

Proteinase K:

___ Bring proteinase K vial to room temperature.

___ Remove stopper and add 1.1 mL of molecular grade water to the proteinase K vial.

___ Replace stopper and incubate at room temperature for 15 minutes, mixing well by inversion.

___ Aliquot into required volumes and store at -20°C. Avoid multiple freeze thaw cycles

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Note: *Ensure that all solutions are at room temperature prior to use.*

Homogenization

1. Add 500 µL of Lysis Buffer, 20 µL of proteinase K, and 10 µL of FoamBlocker into each lysis kit tube.
2. Weigh up to 50 mg of small organism samples and transfer to the buffer-filled tubes. ***Note:*** *Cut into small pieces – larger tissue size may affect complete homogenization with the recommended homogenization setting.*
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 settings.
4. Remove the tubes and visually inspect the samples to confirm complete homogenization (Figure 1). ***Note:*** *If unhomogenized tissue is seen, homogenize for additional 30 seconds.*

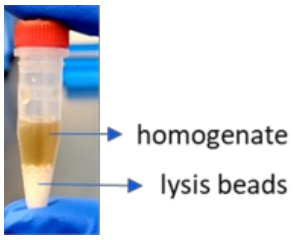

Figure 1: A sample after complete homogenization without any sample chunks.

Extraction

Nucleaic Acid Binding:

1. Prepare fresh microtubes in the tube rack with 200 µL of 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.
5. Add 450 µL (or volume equal to the transferred supernatant from step 4) of Binding Buffer to the supernatant in each tube. Slide the tube rack onto the mixing base and invert to mix the buffer with the homogenate.
6. Vortex the MAGneat beads thoroughly making sure that there are no clumps in the tube.
7. Slide the tube rack off the mixing base and add 30 µL of the beads to each sample tube. Mix tubes by inversion to resuspend the magnetic beads. Allow to stand for 5 minutes, mixing again halfway through.
8. Slide the tube rack onto the magnetic stand and allow to sit for 1-2 minutes, until the magnetic beads separate from the homogenate (Figure 2).
9. Using a pipette, discard the supernatant without disturbing the beads (Figure 3). Remove the tube rack from the magnetic stand.

Figure 2: Magnetic beads separate to the back wall of the tubes, when placed in the magnetic rack.

Figure 3: Using pipette tips, carefully remove the supernatant. If needed, hold the tube to prevent the tube from moving/spinning.

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Washing I:

1. Add 700 µL of Wash Buffer 1 to the tubes and then place the tube rack in the mixing base. Mix thoroughly by inversion, ensuring all beads have been detached from the tube walls and evenly resuspended (Figure 4).
2. Remove the tube rack from the mixing base and place it on the magnetic stand. Allow the magnetic beads to separate.
3. Using a pipette, discard the supernatant. ***Note:*** *Be careful not to disturb the magnetic beads.*
4. Remove the tube rack from the magnetic stand. Add 700 µL of Wash Buffer 2, mix thoroughly by inversion to completely resuspend the beads.
5. Transfer the tube rack to the magnetic stand and allow the magnetic beads to separate.
6. Discard the supernatant. ***Note:*** *Be careful not to disturb the magnetic beads.*
7. Repeat steps 4-6 for a total of 2 washes with Wash Buffer 2.
8. Centrifuge samples at 2,000 x g for 3 minutes to collect the remaining Wash Buffer. Place the tubes on the tube rack.
9. Use a 20 µL pipette to remove the remaining Wash Buffer from the tubes (Figure 5).
10. Leave tube caps open to air dry the magnetic beads at room temperature for 5 minutes. ***Note:*** *Do not dry longer than 10 minutes as this can cause RNA degradation.*

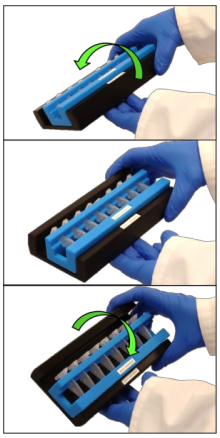

Figure 4: With the tube rack in the mixing base, gently mix the tubes top down (upper panel). Mix until the beads are completely resuspended.

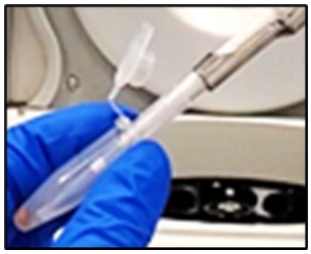

Figure 5: Using the tip of a pipette, carefully remove all of the remaining Wash Buffer that has collected just below the magnetic bead pellet.

Elution and DNase I Treatment:

1. Add 100 µL of RNA Elution Buffer to each tube. Mix by tapping to ensure that the magnetic beads are completely resuspended.
2. 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.
3. 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.*
4. Incubate samples at room temperature for 10 minutes.

Washing II:

1. Add 200 µL of RNA Binding Buffer to each tube. Mix well by inversion, ensuring all beads are resuspended.
2. Add 400 µL of Wash Buffer 2, mix well by inversion.

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3. Place the tube rack onto the magnetic stand and allow the magnetic beads to separate.
4. Discard the supernatant then remove the tube rack from the magnetic stand. ***Note:*** *Be careful not to disturb the magnetic beads.*
5. Add 400 µL of Wash Buffer 2 and mix well by inversion. Ensure beads are detached from the tube walls.
6. Place the tube rack onto the magnetic stand and allow the magnetic beads to separate.
7. Discard the supernatant then remove the tube rack from the magnetic stand. ***Note:*** *Be careful not to disturb the magnetic beads.*
8. Centrifuge samples at 2,000 x g for 3 minutes to collect the remaining Wash Buffer. Place the tubes on the tube rack.
9. Use a 20 µL pipette to remove the Wash Buffer from the tubes. Do not disturb the magnetic beads.
10. Leave caps open to air dry the magnetic beads at room temperature for 5 minutes. ***Note:*** *Do not dry longer than 10 minutes as this can cause RNA degradation.*

Elution II:

1. Add 100 µL of RNA Elution Buffer to each tube. Mix by tapping to ensure that the magnetic beads are completely resuspended.
2. Place the tube rack onto the magnetic stand and allow the magnetic beads to separate.
3. Transfer the clear supernatant containing the RNA into new, labeled microfuge tubes for downstream processing. Be careful not to transfer any magnetic beads.
4. Analyze RNA quality (OD_{260}/OD_{280}) and yield using a NanoDrop™ or other spectrophotometer and gel.
5. Isolated RNA can be stored at -80°C.

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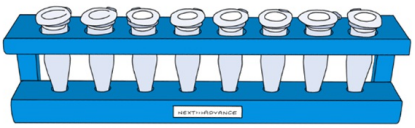
Magnetic Rack Assembly

Using the Magnetic Rack Assembly

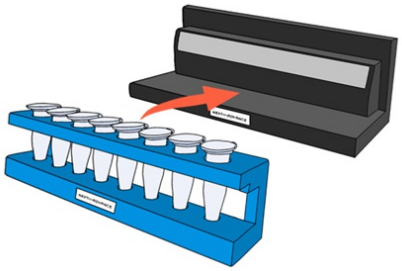
Tube Rack:

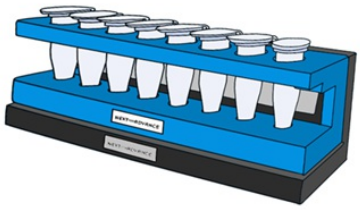
Basic rack that holds up to eight 1.5 mL or 2 mL tubes during every step of procedure. No assembly required.



Magnetic Stand:

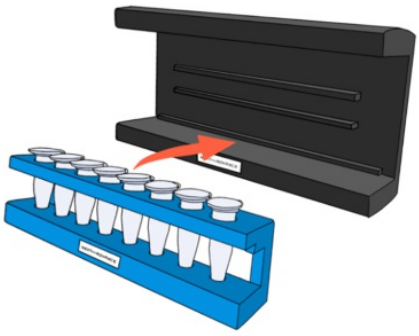
Contains a magnet. Place the tube rack onto the magnetic stand for the bead separation steps.

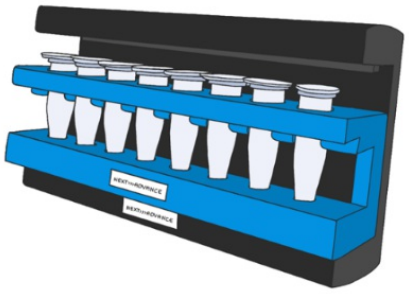




Mixing Base:

Has an overhang to retain tubes in the tube rack during rocking or inversion mixing. Place the tube rack into the mixing base during incubation, binding, and washing steps.





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