RNA Extraction Protocol

Matute Lab
Written By: David Peede
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Materials Needed: Zymo Research Direct-zol RNA MiniPrep Kit

RNaseZAP
TRIZOL
95-100% Ethanol
RNase and DNase-free 1.5 mL Tubes
RNase and DNase-free Pipette Tips

IMPORTANT NOTE: RNase lives on our skin to protect us from RNA viruses, so be careful not to contaminate your samples and if at any point during this protocol you need to touch something (e.g. the Zymo kit, pipettes, reagents, pens, gloves, and/or centrifuge for example) make sure to wipe it down with RNaseZAP beforehand.

1. Create a sterile workplace by cleaning off your bench space with 70% EtOH, followed by RNaseZAP.

2. With gloves on wash one pestle per sample first with soap and water, then with 70% EtOH, and finally with RNaseZAP; after the pestles are appropriately cleaned place them on your sterile workplace.

3. Add 400 µL of TRIZOL to each 1.5 mL tube containing 20-50 frozen *Drosopohila/Aedes* or add 200 µL of TRIZOL to each 1.5 mL tube containing 1-19 frozen *Drosopohila/Aedes* and then homogenize the flies using a clean pestle treated with RNaseZAP until you can no longer recognize any fly parts.

4. Add an equal volume to the amount of TRIZOL added of 95-100% EtOH and mix thoroughly by pipetting.

5. Pipette the mixture into a Zymo-Spin Column in a collection tube, centrifuge at 16,000 x g for 30 seconds, transfer the Zymo-Spin Column into a new collection tube and discard the flow-through.

6. Add 400 µL of RNA Wash Buffer to the Zymo-Spin Column and centrifuge at 16,000 x g for 30 seconds.

7. In a new RNase-free 1.5 mL tube add 5 µL of DNase 1, 75 µL of DNA Digestion Buffer, mix gently by inverting the tube, and add the mixture directly onto the column matrix.

8. Incubate at room temperature for 15 minutes before proceeding to step 9.

9. Add 400 µL of Direct-zol RNA PreWash to the Zymo-Spin Column and centrifuge at 16,000 x g for 30 seconds then discard the flow-through.

10. Repeat step 9.

11. Add 700 µL of RNA Wash Buffer to the Zymo-Spin Column, centrifuge at 16,000 x g for 1 minute, and carefully transfer the Zymo-Spin Column into a new RNase-free 1.5 mL tube.

12. To elute the RNA from the Zymo-Spin Column add 50 µL of RNase and DNase-free water directly onto the column matrix and centrifuge at 16,000 x g for 30 seconds.

13. Store the eluted RNA in the -80.