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Optimized Protocol for RNA Extraction from Insect Samples Using TRIzol Reagent V.1

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We use this protocol and it's working

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Abstract

RNA extraction from insect samples is a crucial step in molecular biology studies aimed at understanding gene expression, functional genomics, and various other applications. This study presents an optimized protocol for RNA extraction from insect tissues using TRIzol reagent. The protocol involves homogenizing insect samples in TRIzol, followed by phase separation with chloroform, RNA precipitation with isopropanol, and washing with ethanol. The final RNA pellet is dissolved in RNase-free water. This method ensures high yield and purity of RNA, suitable for downstream applications such as RT-PCR and RNA sequencing. The efficacy of this protocol was validated through spectrophotometric analysis and agarose gel electrophoresis, demonstrating its reliability and efficiency for extracting RNA from a wide variety of insect species

Materials

1- Insects
2- 75% ethanol
3- TRIzol (Mixture of guanidine thiocyanate and phenol in a single-phase solution)
4- crucible and pistil
5- chloroform
6- Isopropanol
7- Microcentrifuge tubes
8- Centrifuge

Troubleshooting

Steps

1 Cover the crucible with liquid nitrogen to keep the sample cold

2 Place the sample (about 50mg) and macerate, keeping it cold. Remove the wings and macerate insect samples as they have a strong exoskeleton and the presence of chitin

3 Mix the sample with Trizol in the crucible and transfer to a 1.5 mL microtube

4 Add 200 µL of ice-cold chloroform for phase separation. Shake the tube vigorously by hand for a full 15 seconds. Let sit at room temperature for 2-3 minutes

NOTE: You should see the two mixtures separating almost immediately: pink on the bottom and clear on top

5 Add 1mL of Trizol and wait for it to thaw

6 Centrifuge at 12000 rpm for 15 min at 4° C

NOTE: Any denatured proteins might appear as a white interface. Avoiding this interface, carefully move the clear top liquid to a new clean, labeled tube

7 Remove 500 µL of supernatant and add 400 µL of ice-cold isopropanol to precipitate the RNA (always 100 µL less)

8 Leave 10 minutes at room temperature

NOTE: Leaving to precipitate in the -20°C is not recommended because it might cause contaminants to co-precipitate

9 Centrifuge at 12000 rpm for 10 min 4° C

NOTE: You may see the pellet as a clear, gel-like ball at the base of the tube, or a small white smear

10 Remove the isopropanol and wash with 1 mL of 75% ethanol

11 Centrifuge at 9500 rpm for 5 min at 4° C

12 Discard the alcohol and wait for the pellet to dry for 5 to 10 minutes at room temperature

NOTE: Remove as much of the ethanol as possible

13 Resuspend in 30 µL of RNAse-free water depending on the size of the pellet formed

Protocol references

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