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Chapter 25

Trizol-based RNA extraction: Principles, protocol, and applications in molecular biology

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1. Introduction:

Isolation of RNA from biological samples is a crucial step in molecular biology research, enabling the study of gene expression, RNA processing, and regulatory mechanisms. Among the various methods available for RNA extraction, the Trizol reagent-based protocol has emerged as a widely used and highly effective approach (Sambrook and Russell, 2001). Trizol reagent facilitates the simultaneous isolation of RNA, or DNA or proteins from a single sample, making it particularly advantageous for streamlined molecular analyses. Trizol is a monophasic reagent composed of phenol mixed with guanidine isothiocyanate (GITC), designed to disrupt cells and denature macromolecules, facilitating their separation based on density.

When added to biological samples, Trizol efficiently solubilizes cellular components, including RNA, by inactivating ribonucleases (RNases) and forming a stable aqueous phase containing RNA (Srivastava et al., 2022). The versatility and reliability of Trizol-based RNA isolation have made it a cornerstone technique in molecular biology laboratories worldwide. Its compatibility with various sample types, including tissues, cells, and bodily fluids, further its utility in diverse research areas ranging from basic science to clinical diagnostics (Brown, 2010). Moreover, the scalability of the Trizol protocol enables efficient extraction of RNA from small to large sample volumes, accommodating the needs of different experimental designs (Wilson, and Walker, 2010; Rio et al 2010; Tripathi et al.,2013a;b).

2. Materials Required:

1. 1 ml Trizol per sample
2. 50-100 mg tissue sample,
3. Microcentrifuge tubes (sterilized)
4. Micropippette, Centrifuge.

3. Procedure:

1. Begin by placing cells or tissues in Trizol and homogenizing the sample using a pestle.
2. Leave the sample tube five minutes at room temperature.
3. Combine 200 µl of Chloroform with 1 ml of Trizol in a microcentrifuge tube.
4. Gently mix the tube containing the sample.
5. Allow the sample to rest at room temperature for 10 minutes.
6. Centrifuge at 11,000 rpm for 15-20 minutes at 4°C. The sample will separate into three layers, known as phase separation:
    a. The top layer contains a clear aqueous phase, housing RNA.
    b. The middle layer appears as a white cloudy phase, indicating DNA.
    c. The bottom layer, appearing red, contains protein.
7. Extract 80% of the RNA layer from the microcentrifuge tube, leaving 20% of the total volume.
8. Transfer the top layer of RNA, constituting 80% of the sample, into a properly labeled new tube.

3.1. RNA Precipitation:

1. Mix 0.5 ml of isopropanol with 1 ml of Trizol.
2. Gently invert the tubes five times or vortex for 10 seconds.
3. Allow it to rest for 10 minutes at room temperature.
4. Centrifuge the sample at 12,000 rpm for 10 minutes at 4°C.
5. Carefully remove the supernatant using a pipette, ensuring not to disturb the pellet.

3.2. Washing and Purification of RNA:

1. Use 1 ml of cold 75% EtOH (25% DEPC water) for washing.
2. Vortex the pellet.
3. Centrifuge the sample at 9,100 rpm for 5 minutes at 4°C.
4. Carefully remove the liquid on top using a pipette, ensuring not to disturb the solid at the bottom.
5. Allow it to air dry at room temperature for 10-15 minutes.

3.3. Solubilization of the isolated RNA:

1. Set the water bath to 55°C.
2. Mix the RNA pellet with 100 µl of elution buffer and vortex for 15 seconds.
3. Incubate in a water bath for 5 minutes at 55°C.

3.4. Treatment with DNAse:

1. Treat the sample with DNAse enzyme after the above step.
2. Take 10% of the final volume—10 µl—of 10X DNAse buffer out of the refrigerator.
3. Add 1% of the aqueous fraction—1 µl of DNAse I—from the freezer box.
4. Mix by vortexing or by using pipette or and centrifuge.
5. Give it a half hour at 37°C.
6. Add 11 microlitres of DNAse enzyme.
7. Gently vortex for two minutes at room temperature.
8. Centrifuge running for one minute @ 11,000 rpm.
9. Gently move the supernatant, being sure not to disrupt the white pellet, to a new microcentrifuge tube.

3.5. Concentration of RNA:

1. Add 1 µl of linear acrylamide, approximately 1% of the initial volume of the supernatant.
2. Add 10 µl of 5M ammonium acetate, approximately 10% of the initial volume of the supernatant.
3. Include 300 µl of 100% EtOH (1 part Aqueous: 3 parts EtOH).
4. Store in a freezer at -20°C for 1 hour.
5. Centrifuge the sample at 14,000 rpm for 15 minutes at 4°C.
6. Discard the liquid and air dry for 30 minutes.
7. Allow the pellet to air dry for approximately 30-40 minutes.
8. Mix with 50 µl of elution buffer, adjusting the amount based on the pellet size.
9. Mix using a pipette and place on ice. Take readings using a Nanodrop spectrophotometer or check the isolated RNA in Agarose gel.

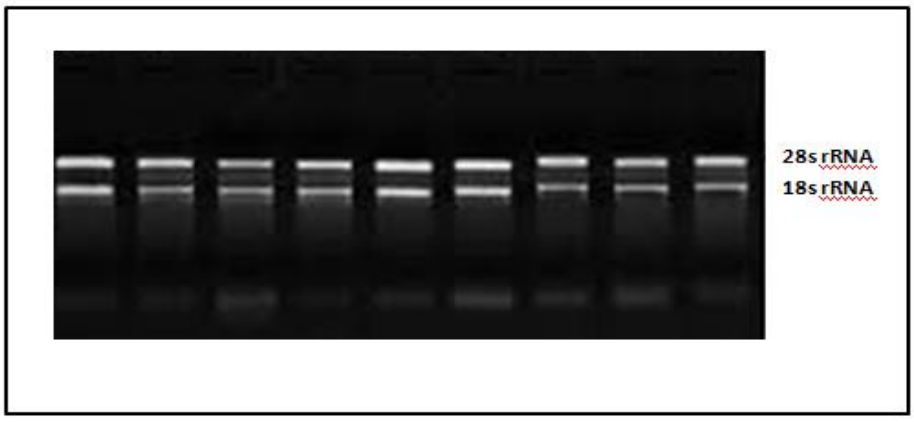

Figure 1: Visualization of RNA on Agarose gel

4. Precautions:

1. Use Trizol reagent in a well-ventilated area and follow safety guidelines for handling chemicals.
2. Ensure all equipment and surfaces are RNase-free to prevent degradation of RNA samples.
3. Work quickly and efficiently to minimize RNA degradation during the isolation process.
4. Use proper personal protective equipment (PPE) such as gloves and lab coat.
5. Choose a suitable source for RNA extraction and ensure it is handled gently to preserve RNA integrity.
6. Use appropriate ratios of Trizol reagent to sample volume as per manufacturer's instructions.
7. Perform homogenization or lysis steps thoroughly and efficiently to maximize RNA yield.
8. Maintain appropriate temperatures during the extraction process to prevent RNA degradation.
9. Perform phase separation carefully and ensure complete separation of RNA-containing phase.
10. Use isopropanol precipitation step to precipitate RNA and minimize co-precipitation of contaminants.
11. Perform DNase treatment to remove genomic DNA contamination, if necessary.
12. Verify the quality and quantity of isolated RNA using spectrophotometry or other suitable methods.
13. Store RNA samples properly (e.g., at -80°C) to maintain integrity and stability.
14. Dispose of used Trizol reagent and contaminated materials according to hazardous waste disposal guidelines.

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