Aug 16, 2024

Protocol for RNA Extraction from Peanut Samples Using Direct-zol™ RNA Miniprep

DOI
dx.doi.org/10.17504/protocols.io.kxygxyq1ol8j/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.kxygxyq1ol8j/v1

External link: https://www.zymoresearch.com/products/direct-zol-rna-miniprep-kits

Protocol Citation: Gabriela Gabriela Paredes 2024. Protocol for RNA Extraction from Peanut Samples Using Direct-zol™ RNA Miniprep. **protocols.io https://dx.doi.org/10.17504/protocols.io.kxygxyq1ol8j/v1**

Manuscript citation:
Manufacturer: Zymo Research Corporation.
Title: *Direct-zol™ RNA Miniprep Instruction Manual*.
Catalog Numbers: R2050, R2051, R2052, R2053.
Publication Date: August 8, 2023.
Website: https://files.zymoresearch.com/protocols/_r2050_r2051_r2052_r2053_direct-zol_rna_miniprep.pdf

Protocol status: Working
We use this protocol and it's working

Created: August 16, 2024

Last Modified: August 16, 2024

Protocol Integer ID: 105732

Keywords: RNA Extraction, Peanut RNA, Direct-zol™ RNA Miniprep, TRIzol®, High Oil Content Samples, Peanut Tissue Homogenization, Bead Beater, RNA Purification, Challenging Samples, Molecular Biology, Gene Expression Analysis, Peanut Seed Research

Disclaimer

This protocol is provided as a guide for RNA extraction using the Direct-zol™ RNA Miniprep kit. Zymo Research Corporation and the authors of this protocol are not responsible for any issues arising from the misuse of this procedure. This protocol is intended for trained professionals and is not for diagnostic use.

Abstract

RNA extraction is a fundamental step in molecular biology, crucial for downstream applications such as qPCR, sequencing, and gene expression analysis. The process of RNA extraction involves the isolation of RNA from cells or tissues, which must be done in a way that preserves the integrity and purity of the RNA. This is especially important because RNA is highly susceptible to degradation by RNases, which are ubiquitous in the environment.
The extraction process typically involves several key steps: cell lysis, removal of proteins and other contaminants, and purification of the RNA. The Direct-zol™ RNA Miniprep kit simplifies these steps by allowing for the direct processing of samples in TRIzol® or similar reagents, which are known for their strong denaturing properties that inactivate RNases and other degradative enzymes. TRIzol® is particularly advantageous for samples with high oil content, such as peanut seeds, as it effectively disrupts the cell membranes and separates nucleic acids from lipids and other cellular components without the need for chloroform, phase separation, or precipitation steps.
Using TRIzol® in combination with the Direct-zol™ RNA Miniprep kit ensures that high-quality total RNA, including small RNAs, can be efficiently extracted even from challenging samples like peanut tissues. This streamlined method reduces the complexity and time required for RNA extraction, making it an ideal choice for researchers working with oil-rich seeds.

Guidelines

- **Aseptic Technique**: Ensure that all steps are performed using aseptic techniques to prevent contamination of RNA samples. Wear gloves, and work in a clean environment, ideally under a laminar flow hood.
- **Sample Homogenization**: Properly homogenize peanut samples using a bead beater to ensure complete cell lysis. Inadequate homogenization can lead to poor RNA yield and contamination.
- **Ethanol Purity**: Use high-purity ethanol (95-100%) in all steps requiring ethanol addition. Lower purity ethanol can lead to inefficient RNA precipitation and lower yields.
- **Temperature Control**: Maintain samples at room temperature during processing. Cold temperatures can cause unwanted precipitation, while high temperatures may degrade RNA.

Materials

- **Direct-zol™ RNA Miniprep Kit** (Catalog Numbers: R2050, R2051, R2052, R2053)
- **TRIzol® or TRI Reagent®** (included in some kit versions)
- **Ethanol (95-100%)**
- **DNase I (included in the kit)**
- **DNase/RNase-Free Water**
- **Sterile microcentrifuge tubes**
- **Peanut samples**
- **Bead beater with appropriate lysis beads**
- **Microcentrifuge**
- **Vortex mixer**

Equipment

- Microcentrifuge (capable of 10,000-16,000 x g)
- Vortex mixer
- Bead beater
- Magnetic stirrer (optional)

Safety warnings

- **RNase Contamination**: RNA is highly susceptible to degradation by RNases. Always wear gloves and use RNase-free materials to prevent contamination.
- **Chemical Safety**: TRIzol® and TRI Reagent® are hazardous chemicals. Handle with care, using appropriate personal protective equipment (PPE) such as gloves and eye protection. Work in a well-ventilated area or under a fume hood.
- **Incomplete Lysis**: Inadequate homogenization can result in incomplete lysis, leading to low RNA yield or contamination. Ensure thorough homogenization using a bead beater with appropriate beads and homogenization cycles.
- **Ethanol Handling**: Ethanol is flammable and should be handled with care. Ensure proper storage and avoid open flames or high temperatures near ethanol.

Ethics statement

This protocol is intended for research purposes only. Researchers should ensure that all procedures are conducted in accordance with institutional guidelines and ethical standards. Proper disposal of hazardous chemicals, such as TRIzol® and ethanol, should be conducted according to local regulations. Additionally, RNA extraction from any biological material should be performed with respect to ethical considerations regarding the source of the material and the intended use of the extracted RNA.

Before start

Before starting the RNA extraction, ensure that all materials and reagents are prepared and that you have completed the following steps:

- **Buffer Preparation**: Add ethanol to the Direct-zol™ RNA PreWash and RNA Wash Buffer concentrates as per the kit instructions.
- **DNase I Reconstitution**: Reconstitute DNase I with DNase/RNase-Free Water and store aliquots as per the kit instructions.
- **Equipment Check**: Verify that the bead beater, centrifuge, and other equipment are functioning properly and set to the appropriate settings.

Preparation Before Starting

1 **Buffer Preparation:**
- Add ethanol to the Direct-zol™ RNA PreWash concentrate and RNA Wash Buffer concentrate according to the kit instructions.
- Reconstitute the lyophilized DNase I with DNase/RNase-Free Water as per the instructions provided in the kit.

2 **Sample Preparation**:
- **Tough-to-lyse samples (e.g., peanut tissues)**: For peanut tissues, which are challenging due to their high oil content, homogenize the samples using a bead beater. Use a sufficient amount of TRIzol® or TRI Reagent® (at least 800 µl) to ensure complete lysis.

3 **Bead Beater Instructions:**
- Use 2.0 mm beads for effective disruption of peanut tissues.
- Add the tissue sample and beads to a suitable tube, then add TRIzol®.
- Homogenize the sample at high speed for 30-60 seconds. Allow the sample to cool for 1-2 minutes, then repeat the homogenization for another 30-60 seconds.
- If the lysate appears viscous or if there is visible precipitation, add additional TRIzol® (100-200 µl) and continue homogenizing until the lysate is smooth and homogeneous.

  After homogenization, centrifuge the sample to remove particulate debris and transfer the supernatant to a new RNase-free tube.

What to Do if Lysis is Incomplete or Precipitation is Observed

4 **Incomplete Lysis:**
- Ensure that the sample is thoroughly homogenized by increasing the homogenization time or repeating the bead beating cycle.
- If the sample remains viscous, indicating incomplete lysis, add additional TRIzol® (100-200 µl) and homogenize again.
- Use a larger volume of TRIzol® initially to ensure complete lysis, especially for samples with high lipid content like peanuts.

5 **Precipitation:**
- Precipitation can occur if the sample is not fully homogenized or if the TRIzol® is not sufficient to dissolve all components.
- To resolve this, add more TRIzol® (up to double the original volume) and re-homogenize the sample.
- Ensure that the sample is kept at room temperature during processing to prevent premature precipitation of nucleic acids.

RNA Extraction Protocol

6 **Lysis and Binding:**
Add an equal volume of ethanol (95-100%) to the homogenized sample in TRIzol® or TRI Reagent®. Mix thoroughly by vortexing.

Example: For 400 µl of the sample, add 400 µl of ethanol.

7 **RNA Binding:**
Transfer the mixture into a Zymo-Spin™ IICR Column in a collection tube. Centrifuge at 10,000-16,000 x g for 30 seconds.

Discard the flow-through and place the column in a new collection tube.

8 **DNase I Treatment (Optional, Recommended)**:
- Add 400 µl of RNA Wash Buffer to the column and centrifuge.
- In a separate RNase-free tube, prepare the DNase I mix by adding 5 µl of DNase I (6 U/µl) to 75 µl of DNA Digestion Buffer. Mix gently.
- Add the DNase I mix directly to the column matrix. Incubate at room temperature (20-30°C) for 15 minutes.

9 **Column Washing:**
After the incubation, add 400 µl of Direct-zol™ RNA PreWash to the column and centrifuge. Discard the flow-through.

Repeat the wash step with another 400 µl of Direct-zol™ RNA PreWash.

Add 700 µl of RNA Wash Buffer to the column and centrifuge for 1 minute to ensure the complete removal of any residual buffer.

10 **RNA Elution**:
- Carefully transfer the column to a new RNase-free tube.
- Add 50 µl of DNase/RNase-Free Water directly to the column matrix and centrifuge to elute the RNA.
- For a more concentrated RNA sample, use a lower elution volume (≥ 25 µl).

Post-Extraction Steps

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- **Storage**: The eluted RNA can be used immediately for downstream applications or stored at -80°C for long-term storage.
- **Quality Check**: Assess RNA quality and quantity using a spectrophotometer (A260/A280 & A260/A230 ratios) and/or agarose gel electrophoresis.