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GeneJET™ Plant RNA Purification Mini Kit

USER GUIDE

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN1001381 A (English)

Revision	Date	Description
A	31 March 2025	• Initial release with new publication number. Supersedes publication number MAN0012668, Rev. 7. • Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • The contents and storage table was updated to include additional instructions for storage of purification columns.

The information in this guide is subject to change without notice.

Contents

1 Product information

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

GeneJET™ Plant RNA Purification Mini Kit is designed for rapid and efficient purification of high-quality total RNA from a wide variety of plant species and tissue types. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation.

The standard procedure takes less than 20 minutes following cell lysis. RNA yields vary between different species, tissues, and ages of tissue sample. The purified high-quality RNA can be used in a wide range of downstream applications such as RT-PCR, RT-qPCR, Northern blotting, and other RNA-based analysis. For typical total RNA yields from various sources, see Table 1.

Technology overview

Cells are lysed in a buffer containing guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous RNases. The lysate is then mixed with ethanol and loaded on the purification column. The chaotropic salt and ethanol cause RNA binding to a silica membrane when the lysate is spun through the column. Impurities are effectively removed in subsequent wash steps. Pure RNA is then eluted under low ionic strength conditions with the nuclease-free water provided in the kit.

Typical RNA yield

Table 1 Typical total RNA yields from 50 mg of fresh tissue from various sources

Plant	Tissue	RNA yield
Arabidopsis thaliana	leaf	17–20 µg
Nicotiana tabacum	leaf	17–21 µg
Tomato	leaf	50–53 µg
Dandelion	leaf	30–33 µg
Spinach	leaf	25–30 µg
Corn	leaf	25–30 µg
	seeds	6–7 µg
Sugar-cane	leaf	4–5 µg
Rape	leaf	25–30 µg
	root	20–22 µg
	seeds	40–42 µg
Onion	leaf	10–11 µg
Lettuce	leaf	10–11 µg
Lucerne	leaf	35–40 µg
Cucumber	fruit	40–45 µg
Lemon	leaf	5–7 µg
Rice	leaf	20–25 µg
Wheat	leaf	60–65 µg
Sunflower	stalk	10–11 µg
	seeds	10–12 µg
Sugar-beet	roots	6–7 µg
Soy	seeds	5–7 µg

Contents and storage

Table 2 Components of the GeneJET™ Plant RNA Purification Mini Kit

Component	Cat. No. K0801 (50 reactions)	Cat. No. K0802 (250 reactions)	Storage
Plant RNA Lysis Solution	40 mL	200 mL	15–25°C
Wash Buffer I (concentrated)	40 mL	200 mL	
Wash Buffer II (concentrated)	30 mL	2 × 100 mL	
Water, nuclease-free	30 mL	125 mL	
GeneJET™ Genomic RNA Purification Columns and Collection Tubes	50	250	15–25°C For better long-term performance store at 2°C to 8°C.
Collection Tubes, 2 mL	50	250	15–25°C
Collection Tubes, 1.5 mL	50	250	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Adjustable micropipettor	**MLS**
Vortex mixer, or equivalent	**MLS**
Thermo heating-blocks or water bath (adjustable to 56°C)	**MLS**
Centrifuge capable of ≥20,000 × *g* for 1.5 mL microcentrifuge tubes	**MLS**
Mortar and pestle or grinding mill	**MLS**
Microcentrifuge tubes (1.5 mL) with screw caps	**MLS**
RNase-free pipette tips (sterile)	**MLS**
Disposable gloves	**MLS**
Ethanol, 96–100% (molecular biology grade)	**MLS**
Dithiothreitol (DTT)	R0861
(Optional) DNase I, RNase-free (1 U/µL)	EN0521

(continued)

Item	Source
(Optional) RiboLock RNase Inhibitor	EO0381
(Optional) RapidOut DNA Removal Kit	K2981

Procedural guidelines

IMPORTANT! Tightly seal the bag containing GeneJET™ Genomic RNA Purification Columns after each use.

- Wear gloves when handling the Plant RNA Lysis Solution and Wash Buffer I as these solutions contain irritants and are harmful if contacted with skin, inhaled, or swallowed.
- Perform all purification steps after sample homogenization at room temperature (15–25°C).
- Keep the RNA on ice after extraction and while working with it.
- Store the extracted RNA at -20°C or -70°C. For long term stability, keep the RNA at -70°C.

Recommendations for avoiding RNA contamination

RNA purity and integrity is important for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Care must be taken not to introduce RNases into RNA preparation, especially during the column wash with Wash Buffer II and elution steps.

- Wear gloves when handling reagents and RNA samples, as skin is a common source of RNases. Change gloves often.
- Use sterile, disposable RNase-free pipette tips.
- Use appropriate reagents to remove RNase contamination from non-disposable items and work surfaces.
- Keep all kit components tightly sealed when not in use. After usage, close bottles immediately.

Before first use of the kit

1. Add the indicated volume of ethanol (96–100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) before first use.

Component	Cat. No. K0801 (50 reactions)		Cat. No. K0802 (250 reactions)	
Wash Buffer (concentrated)	Wash Buffer I (40 mL)	Wash Buffer II (30 mL)	Wash Buffer I (200 mL)	Wash Buffer II (100 mL)
Ethanol (96–100%)	2.1 mL	30 mL	10.5 mL	100 mL

2. Mark the checkbox on the bottlecap to indicate that ethanol has been added to the bottle.

Before each use of the kit

1. Check Plant RNA Lysis Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.
2. Determine the amount of Plant RNA Lysis Solution required to process the samples.
3. Add 10 µL of 2 M DTT to each 500 µL of Plant RNA Lysis Solution required.

2 Methods

Prepare tissue samples

Most plant tissue samples can be homogenized using the standard protocol (see page 9), but alternate protocols are provided for lignified, polyphenol-rich samples (see page 10), and soybean seeds (see page 11).

- Use up to 100 mg of plant tissue for each sample preparation.
- 500 µL of Plant RNA Lysis Solution is required to process each tissue sample.
- Transfer the ground tissue to the Plant RNA Lysis Solution as quickly as possible to avoid RNA degradation.
- All ground material must be thoroughly mixed with the Plant RNA Lysis Solution. RNA degradation can occur in particles that are left to dry on the walls of the tube.

Homogenize plant tissues

1. Add 500 µL of Plant RNA Lysis Solution supplemented with DTT see page 8) into a 1.5 mL microcentrifuge tube.
2. Use up to 100 mg of fresh or frozen tissue or up to 20 mg of lyophilized tissue. Grind the material using one of the following methods:
   - Place up to 100 mg of tissue into liquid nitrogen in a mortar, then grind thoroughly using a mortar and pestle.
   - Place up to 100 mg of tissue into a vial containing stainless steel beads (grinding mill). The vial and beads should be precooled with liquid nitrogen. The setup of the mechanical disruption depends on the tissue type.
3. Immediately transfer the tissue powder into a 1.5 mL microcentrifuge tube containing 500 µL of Plant RNA Lysis Solution. See “Prepare tissue samples” on page 9.

   Note: Ground tissue can be used immediately in the RNA isolation protocol or stored at -70°C until use.
4. Vortex for 10–20 seconds to mix thoroughly.
5. Incubate for 3 minutes at 56°C.
6. Centrifuge at ≥20,000 × *g* for 5 minutes.
7. Proceed to step 1 of “Purify RNA samples” on page 12.

Homogenize lignified, polyphenol-rich plant tissues

This protocol describes how to process woody, lignified, or polyphenol-rich samples such as branches, twigs, needles, wax-coated leaves, or wheat flour for RNA purification.

1. Add 500 µL of Plant RNA Lysis Solution supplemented with DTT to a 2% (w/v) final concentration into a 1.5 mL microcentrifuge tube (see page 8).
2. Add 10 mg of polyvinylpyrrolidone (PVP) per 500 µL of Plant RNA Lysis Solution.
3. Grind the tissue using one of the following methods:
   - Place up to 100 mg of tissue into liquid nitrogen in a mortar, then grind thoroughly using a mortar and pestle.
   - Place up to 100 mg of tissue into a vial containing stainless steel beads (grinding mill). The vial and beads should be precooled with liquid nitrogen. The setup of the mechanical disruption depends on the tissue type.
4. Transfer the tissue powder immediately into the 1.5 mL microcentrifuge tube containing 500 µL of Plant RNA Lysis Solution with DTT and PVP.
5. Vortex for 10–20 seconds to mix thoroughly.
6. (*Optional*) For tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube, then vortex for 1 minute.
7. Incubate for 5 minutes at 56°C.
8. Centrifuge at ≥20,000 × g for 5 minutes.
9. Proceed to step 1 of “Purify RNA samples” on page 12.

Homogenize soybean seeds

This protocol describes how to process soybean seeds for RNA purification.

1. Add 500 µL of Plant RNA Lysis Solution supplemented with DTT and NaCl at a 2 M final concentration (see page 8) into a 1.5 mL microcentrifuge tube.
2. Grind the material using one of the following methods:
   - Place up to 100 mg of tissue into liquid nitrogen in a mortar, then grind thoroughly using a mortar and pestle.
   - Place up to 100 mg of tissue into a vial containing stainless steel beads (grinding mill). The vial and beads should be precooled with liquid nitrogen. The setup of the mechanical disruption depends on the tissue type.
3. Immediately transfer the tissue powder into the 1.5 mL microcentrifuge tube containing 500 µL of Plant RNA Lysis Solution supplemented with DTT and NaCl.
4. Vortex for 10–20 seconds to mix thoroughly.
5. (*Optional*) For tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube, then vortex for 1 minute.
6. Incubate for 3 minutes at 56°C.
7. Centrifuge at ≥20,000 × *g* for 5 minutes.
8. Proceed to step 1 of “Purify RNA samples” on page 12.

Purify RNA samples

1. Transfer the supernatant (usually 450–550 µL) to a fresh microcentrifuge tube.
2. Add 250 µL of 96% ethanol, then mix by pipetting.
3. Transfer the prepared mixture to a purification column inserted in a collection tube.
4. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flowthrough solution, then reassemble column and collection tube.

   Note: Close the bag with purification columns tightly after each use.

5. Add 700 µL of Wash Buffer I supplemented with ethanol (see page 7) to the purification column.
6. Centrifuge at 12,000 × g for 1 minute. Discard the flow-through and collection tube.
7. Place the purification column into a clean 2 mL collection tube.
8. Add 500 µL of Wash Buffer II supplemented with ethanol (see page 7) to the purification column.
9. Centrifuge at 12,000 × g for 1 minute. Discard the flow-through solution, then reassemble column and collection tube.
10. Repeat step 8 and step 9.
11. Centrifuge the empty column at ≥20,000 × g for 1 minute. Discard the collection tube containing the flow-through solution, then transfer the purification column to a RNase-free 1.5 mL collection tube.
12. To elute the RNA, add 50 µL of nuclease-free water to the center of the purification column membrane. Centrifuge at 12,000 × g for 1 minute.

    Note: Repeat the elution step when yields >30 µg are expected.

13. Discard the purification column.

Use the purified RNA immediately in downstream applications, store at -20°C until use, or for prolonged storage (more than 1 month) storage at -70°C is recommended.

A Troubleshooting

Observation	Possible cause	Recommended action
Low RNA yield	Insufficient homogenization of plant material.	To disrupt the cell wall, it is important to homogenize the sample thoroughly until it is ground to fine powder.
	Excess sample used during lysate preparation.	Decrease the amount of starting material. Do not use more than 100 mg of plant tissue per column.
	Ethanol was not added to the lysate.	Ensure ethanol was added to the lysate before applying the sample to the purification column.
	Ethanol was not mixed properly with the lysate.	After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting.
	Ethanol was not added to Wash Buffers.	Ensure ethanol was added to Wash Buffer I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation.
	DTT was not added to Plant RNA Lysis Solution.	DTT should be added fresh to an aliquot of the Plant RNA Lysis Solution prior to the purification procedure.
	Sample may be old or degraded.	If possible, use young leaves or tissues.
Purified RNA is degraded	RNase contamination.	To avoid ribonuclease contamination, wear gloves during every procedure involving RNA extraction, purification and subsequent applications. Change gloves often. Use sterile, disposable RNase-free pipette tips. Treat non-disposable items and work surfaces with solutions designed to eliminate RNases.
	Inappropriate sample storage conditions.	Flash-freeze and homogenize plant sample in liquid nitrogen. Immediately transfer homogenized powder into Plant RNA Lysis Solution.
		Purified RNA should be used immediately in the downstream applications or stored at -20°C for later use. For prolonged storage (more than 1 month), freezing at -70°C is recommended.
Inhibition of downstream enzymatic reactions	Purified RNA contains residual ethanol.	If residual solution is seen in the purification column after washing the column with Wash Buffer II, empty the collection tube then centrifuge the column at ≥20,000 × *g* for an additional 1 minute.
	Purified RNA contains residual salt.	Use the correct order for the Wash Buffers. Always wash the purification column with Wash Buffer I first, then proceed to washing with Wash Buffer II.
Column clogging	Clarified supernatant contaminated with cell debris.	Make sure not to transfer any pelleted precipitate from the lysate clarifying centrifugation step onto spin column.

Observation	Possible cause	Recommended action
Column clogging *(continued)*	Low temperature during centrifugation.	At low temperatures nucleic acids can precipitate and clog the column. Maintain 20–25°C temperature during centrifugation steps.
DNA contamination	DNA rich plant sample.	The amount of gDNA co-purified with the sample RNA varies with the plant species used and can be substantial. To completely remove gDNA, digest the RNA preparation with DNase I, RNase-free (1 U/µL).

B

Supplemental procedure

Remove genomic DNA from RNA preparations using DNase I

1. Add the following reagents to an RNase-free tube.

Reagent	Amount
RNA [1]	1 µg
10X reaction buffer with $MgCl_2$	1 µL
DNase I, RNase-free (1 U/µL) [2]	1 µL
(*Optional*) RiboLock RNase Inhibitor (40 U/µL) [3]	0.25 µL
Water, nuclease-free	to 10 µL

[1] The recommended final concentration of RNA is 0.1 µg/µL.
[2] Do not use more than 1 u of DNase I per 1 µg of RNA.
[3] RiboLock RNase Inhibitor can be added at 1 U/µL to prevent RNA degradation.

Note: Reaction volumes can be scaled to accommodate larger amounts of DNA or when working with diluted RNA samples, however, it is important to maintain the 1 u DNase/µg RNA ratio.

2. Incubate at 37°C for 30 minutes.
3. Add 1 µL 50 mM EDTA, then incubate at 65°C for 10 minutes to inactivate the DNase I.
   EDTA is required because RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent.
   When scaling reaction volumes, it is important to maintain the ~5 mM final EDTA concentration.

Use the prepared RNA directly for downstream applications.

Remove genomic DNA from RNA preparations using a kit

The RapidOut DNA Removal Kit is designed for convenient removal of gDNA from RNA sample and subsequent removal of DNase I in a simple two-step procedure. The kit contains recombinant DNase I, RNase-free, and a proprietary DNase Removal Reagent for efficient DNase I removal. For ordering information see “Required materials not supplied” on page 6.

C Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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