MagMAX™ Plant RNA Isolation Kit

USER GUIDE

High-throughput purification of RNA from plant tissues

Catalog Numbers A33784 and A33899

Publication Number MAN0016311

Revision A.0

ThermoFisher SCIENTIFIC

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0016311

Revision	Date	Description
A.0	08 December 2016	New document

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

Contents

Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The MagMAX™ Plant RNA Isolation Kit is designed for rapid, high-throughput purification of high-quality RNA from a wide variety of plant species. The kit uses MagMAX™ magnetic beads technology, ensuring reproducible recovery of RNA that is suitable for a broad range of applications, including TaqMan® Gene Expression Assays.

The procedures of the MagMAX™ Plant RNA Isolation Kit describe the isolation of RNA from 10–50 mg of plant sample, optimized for use with the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (96DW; 96-well deep well setting), the KingFisher™ Duo Prime Magnetic Particle Processor (12-well deep well setting), or for manual sample processing in a microcentrifuge tube. See Table 1 for typical total RNA yields from various sources.

Table 1 Typical RNA yields from 50 mg of various plants

Source	RNA yield
Arabidopsis thaliana leaves	4–6 µg
Bell pepper leaves	25 µg
Canola/rapeseed leaves	11–28 µg
Corn leaves	13–20 µg
Green onion leaves	4–10 µg
Lemon leaves	13–17 µg
Oat leaves	10–25 µg
Orange leaves	16 µg
Pine needles	2–14 µg
Spinach leaves	15–16 µg
Tomato leaves	9–42 µg
Wheatgrass leaves	23–47 µg

Contents and storage

Table 2 MagMAX™ Plant RNA Isolation Kit (Cat. Nos. A33784 and A33899)

Contents	Cat. No. A33784 (96 reactions)	Cat. No. A33899 (384 reactions)	Storage
Lysis Buffer	72 mL	2 × 140 mL	15 to 25°C
RNA Binding Beads[1]	2 × 1.4 mL	10.6 mL	2 to 8°C
DNase I (lyophilized)[2]	1 vial	4 vials	–25°C to –15°C
DNase I Reconstitution Buffer	1 mL	2 × 1 mL	
Manganese Chloride Solution	3 × 1 mL	9 × 1 mL	
2X DNase I Buffer	12 mL	45 mL	
Wash Solution 1 Concentrate[2]	110 mL	3 × 110 mL	15 to 25°C
Wash Solution 2 Concentrate[2]	50 mL	3 × 50 mL	
Rebinding Buffer	20 mL	70 mL	
Nuclease-Free Water	30 mL	125 mL	

[1] Do not freeze the RNA Binding Beads.
[2] See "Before first use of the kit" on page 11.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 3 Materials required for RNA purification (all methods)

Item	Source
Equipment	
Heat block, water bath, or thermomixer at 56°C	MLS
Centrifuge capable of ≥16,000 × *g* for microcentrifuge tubes	MLS
Adjustable micropipettors	MLS
Vortex mixer, or equivalent	MLS
Tubes, plates, and other consumables	
(Optional) Homogenizer	12183026
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	AM12450

Item	Source
(For purification in plate format) MicroAmp™ Clear Adhesive Film	4306311
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
Reagents	
Ethanol, 96–100% (molecular biology grade)	MLS
Dithiothreitol (DTT)	MLS
(For purification from soy beans) Sodium chloride (NaCl)	MLS
One of the following for purification from woody, lignified, and polyphenol-rich samples:	
Polyvinylpyrrolidone (PVP40) powder	MLS
Plant RNA Isolation Aid	AM9690

Table 4 Additional materials required for manual disruption

Item	Source
Equipment	
Mortar and pestle	MLS
Spatula	MLS
Reagents	
Liquid nitrogen	MLS

Table 5 Additional materials required for mechanical disruption

Item	Source
Equipment	
Centrifuge capable of 3000–4000 × *g* with swinging-bucket rotor for 96-well deep well plates	MLS
Homogenizer, one of the following:	
Fisher Scientific™ Bead Mill 24 Homogenizer, or equivalent	Fisher Scientific 15-340-163
Fisher Scientific™ Bead Mill 4 Homogenizer, or equivalent	Fisher Scientific 15-340-164
Fisher Scientific™ PowerGen™ High-Throughput Homogenizer, or equivalent	Fisher Scientific 02-215-503
Rotor-stator homogenizer with generator	MLS

Item	Source
Other accessories	
Plates or tubes recommended by the homogenizer's manufacturer	MLS
Stainless steel beads (for use with a bead mill or high-throughput homogenizer)	MLS

Table 6 Additional materials required for automated purification

Item	Source
Magnetic particle processor, one of the following:	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Plates and combs	
96 deep-well plates, one of the following:	
MagMAX™ Express-96 Deep Well Plates	4388476
KingFisher™ Deepwell 96 Plate	95040450
96-well standard plates, one of the following:	
MagMAX™ Express-96 Standard Plates	4388475
KingFisher™ 96 KF microplate (200 µL)	97002540
Tip comb, compatible with the magnetic particle processor used:	
MagMAX™ Express-96 Deep Well Tip Combs[1]	4388487
KingFisher™ 96 Tip Comb for DW Magnets	97002534
KingFisher™ Duo 12-Tip Comb, for Microtiter 96 Deepwell plate	97003500
Other consumables	
(For KingFisher™ Duo Prime Magnetic Particle Processor) KingFisher™ Duo Elution Strip	97003520
Reagent reservoirs	MLS
Equipment	
Multi-channel micropipettors	MLS

[1] Compatible with the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.

Table 7 Additional materials required for manual purification

Item	Source
Equipment	
Thermomixer at 37°C with associated block (1.5 mL)	MLS
Other accessories	
DynaMag™–2 magnet	12321D

If needed, download the KingFisher™ Flex or Duo Prime program

The programs required for this protocol are not pre-installed on the KingFisher™ Flex Magnetic Particle Processor 96DW or on the KingFisher™ Duo Prime Magnetic Particle Processor.

1. On the MagMAX™ Plant RNA Isolation Kit product web page, scroll down to the **Product Literature** section.
2. Right-click on the appropriate program file to download the program to your computer:
   - **KingFisher™ Flex Magnetic Particle Processor 96DW: A33784_Plant_RNA_Flex**
   - **KingFisher™ Duo Prime Magnetic Particle Processor: A33784_Plant_RNA_Duo**
3. Select **Save link as...** to download to your computer.
4. Refer to *Thermo Scientific™ KingFisher™ Flex User Manual* (Pub. No. N07669) and *BindIt™ Software User Manual* (Pub. No. N07974) for instructions for installing the program on the instrument.

Methods

Procedural guidelines

General procedural guidelines

- Perform all steps at room temperature (15–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Always change pipette tips between liquid transfers to avoid cross-contamination. We recommend the use of aerosol-resistant pipette tips.
- When working with RNA:
  - Wear clean gloves and a clean lab coat.
  - Change gloves whenever you suspect that they are contaminated.
  - Open and close all sample tubes carefully. Avoid splashing or spraying samples.
  - Use RNase-free pipette tips to handle the kit reagents, and avoid putting used tips into the reagent containers.
  - Clean lab benches and equipment periodically with an RNase decontamination solution, such as RNase*Zap*™ Solution (Cat. No. AM9780).
- Volumes for reagent mixes are given per sample. We recommend that you prepare master mixes for larger sample numbers. To calculate volumes for master mixes, refer to the per-sample volume and add 5–10% overage.

Guidelines for sample handling and homogenization

- To minimize RNA degradation, avoid repeated freezing and thawing of the samples and perform isolation from fresh material, or material that has been immediately frozen and stored at –80°C.
- Appropriate sample storage is essential for reproducibility and high RNA quality and yields. Quality and yields of RNA may vary depending on sample age, type of sample, and storage conditions.
- The lysis procedure is most effective with well homogenized samples.

  Appropriate methods include:
  - Grinding into a powder with a mortar and pestle in the presence of liquid nitrogen
  - Homogenizing with a rotor-stator homogenizer
  - Homogenizing with a bead mill or high-throughput homogenizer in the presence of stainless steel beads
- Clarified lysates can be stored at 2–8°C or at –20°C for up to 5 days before adding the RNA Binding Beads and the ethanol. Ensure that samples are completely thawed and mixed thoroughly before use.

Before you begin

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 110 mL of 96–100% ethanol to each bottle of Wash Solution 1 Concentrate, mix, check the box on the bottle label to indicate that this step has been completed, then store at room temperature.
  - Add 200 mL of 96–100% ethanol to each bottle of Wash Solution 2 Concentrate, mix, check the box on the bottle label to indicate that this step has been completed, then store at room temperature.

- Prepare the DNase I Solution:
  1. Add 440 µL of DNase I Reconstitution Buffer to each vial of DNase I (lyophilized).
  2. Incubate for 5 minutes.
     Occasional gentle rotation of the vial helps to dissolve the DNase I, but avoid forceful mixing. Do not vortex.
  3. Store at –20°C.

Before each use of the kit

- Check all the solutions in the kit for salt precipitation. Dissolve any precipitates by warming the solution at 37°C, then equilibrate to room temperature.

- Prepare a fresh aliquot of Lysis Buffer supplemented with DTT according to the following table.

Component	Volume
Lysis Buffer	600 µL
2M DTT	12 µL
Total Lysis Buffer with DTT	**612 µL**

Note: Only 600 µL of the Lysis Buffer with DTT is required for each sample disruption.

- Resuspend the RNA Binding Beads well by vortexing.

- Set heat block, water bath, or thermomixer to 56°C.

- **For manual purification only:** Set up an extra thermomixer to 37°C and 350 rpm (for the DNase I treatment).

- Add additional supplements to the Lysis Buffer if needed.

  Note: Supplement only the volume of Lysis Buffer that is needed for immediate isolation. We recommend using the supplemented Lysis Buffer only during the day that it is prepared.

  - **For soy beans:** Add NaCl to the Lysis Buffer with DTT at a 2M final concentration of NaCl.
  - **For woody, lignified, or polyphenol-rich samples (branches, twigs, needles, and wax-coated leaves):**
    – Add PVP40 to the Lysis Buffer with DTT at a 2% (w/v) final concentration of PVP40.

      Note: To maximize the effectiveness of PVP40, we recommend adding the PVP40 from a stock solution (for example, 20% w/v) instead of dissolving the powder directly into the Lysis Buffer with DTT.

    – Alternatively, supplement the Lysis Buffer with DTT with the Plant RNA Isolation Aid according to the following table.

Component	Volume per sample
Lysis Buffer with DTT	525 µL
Plant RNA Isolation Aid	75 µL
Total Supplemented Lysis Buffer	**600 µL**

Disrupt the tissue

- To disrupt the tissue manually, proceed to "Disrupt the sample manually" on page 12.
- To disrupt the tissue using a rotor-stator, bead mill or high-throughput homogenizer, proceed to "Disrupt the sample mechanically" on page 13.

Disrupt the sample manually

We recommend using 10–50 mg of fresh plant samples or 10–20 mg of seed samples.

1. Add liquid nitrogen to a clean mortar.
2. Freeze the plant tissue by placing it in the liquid nitrogen in the mortar.
3. Grind the tissue thoroughly using a clean pestle, then allow the liquid nitrogen to evaporate.
4. Add 600 µL of Lysis Buffer with DTT to a 1.5-mL microcentrifuge tube.
5. Transfer up to 50 mg of homogenized sample into the tube containing Lysis Buffer with DTT, then mix thoroughly by vigorously vortexing for 10–20 seconds.

   IMPORTANT! Transfer the ground tissue to the Lysis Buffer with DTT as quickly as possible to avoid RNA degradation.

6. Centrifuge briefly to collect the liquid at the bottom of the tube.
7. Incubate at 56°C for 5 minutes.

8. Centrifuge for 10 minutes at maximum speed (≥ 16,000 × *g*) to clear the plant lysate.

   Alternatively, pass the lysate through a Homogenizer cartridge (Cat. No. 12183026) for 2 minutes at 12,000 × *g*.

9. Transfer 400 µL of the supernatant to a clean 1.5-mL microcentrifuge tube (manual purification) or to the well of a 96-deep well plate (automated purification).

STOPPING POINT You can store the clarified lysates as indicated in "General procedural guidelines" on page 10.

Proceed with the RNA purification.

- For automated purification using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to "Purify RNA using the KingFisher™ Flex Magnetic Particle Processor 96DW" on page 14.
- For automated purification using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to "Purify RNA using the KingFisher™ Duo Prime Magnetic Particle Processor" on page 16.
- For manual purification, proceed to "Purify RNA manually" on page 18.

Disrupt the sample mechanically

Plant tissue can be homogenized with a rotor-stator, bead mill, or high-throughput homogenizers. We recommend bead mill or high-throughput homogenizers paired with stainless steel beads. When using beads for homogenization, ensure that the correct tubes are used, which typically have thicker walls and a screw cap with an o-ring. High-throughput homogenizers offer an appropriate method for handling 96 samples simultaneously. For low-throughput homogenization, it is possible to use 1.5– or 2.0–mL microcentrifuge tubes.

For mechanical disruption with bead mill or high-throughput homogenizers, we recommend using:

- 10–50 mg of plant sample in single microtubes
- 10–20 mg of plant sample in racked 96-well collection microtubes
- 10–20 mg of plant seeds as starting material in racked 96-well collection or single microtubes

For mechanical disruption with a rotor-stator homogenizer, we recommend using:

- Up to 50 mg of plant sample in a single microcentrifuge tube
- Up to 20 mg of plant seeds as starting material in a single microcentrifuge tube

1. *(Optional)* Add a stainless steel bead to a tube if required for your method.
2. Add 600 µL of Lysis Buffer with DTT to the tube.
3. Place the plant tissue in the tube.
4. Homogenize the sample according to the manufacturer recommendations.
5. After sample homogenization, incubate the sample at 56°C for 5 minutes.

6. Centrifuge for 10 minutes at maximum speed (≥ 16,000 × *g* for tubes or 3000–4000 × *g* for plates) to clear the plant lysate.

7. Transfer 400 µL of the supernatant to a clean 1.5-mL microcentrifuge tube (manual purification) or to the well of a 96-deep well plate (automated purification).

STOPPING POINT You can store the clarified lysates as indicated in "General procedural guidelines" on page 10.

Proceed with the RNA purification.

- For automated purification using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to "Purify RNA using the KingFisher™ Flex Magnetic Particle Processor 96DW" on page 14.
- For automated purification using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to "Purify RNA using the KingFisher™ Duo Prime Magnetic Particle Processor" on page 16.
- For manual purification, proceed to "Purify RNA manually" on page 18.

Purify RNA using the KingFisher™ Flex Magnetic Particle Processor 96DW

Set up the processing plates

Ensure that ethanol has been added to the Wash Solution Concentrates (see "Before first use of the kit" on page 11).

1. Prepare the DNase I Master Mix for the number of samples to be processed according to the following table.

   IMPORTANT! For best results, prepare the DNase I Master Mix just prior to use to avoid extended periods of time at room temperature.

Component	Volume per sample
2X DNase I Buffer	100 µL
DNase I Solution	4 µL
Manganese Chloride Solution	20 µL
Nuclease-Free Water	76 µL
Total DNase I Master Mix	**200 µL**

2. Add processing reagents to the wells of 96-well plates as indicated in the following table.

Table 8 Processing plates setup

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash 1_1 Plate	2	Deep Well	Wash Solution 1	700 µL
DNase I Plate	3	Deep Well	DNase I Master Mix	200 µL
Wash 1_2 Plate	4	Deep Well	Wash Solution 1	700 µL
Wash 2_1 Plate	5	Deep Well	Wash Solution 2	700 µL
Wash 2_2 Plate	6	Deep Well	Wash Solution 2	700 µL
Elution Plate	7	Standard	Nuclease-Free Water	100 µL
Tip Plate	8	Place a KingFisher™ 96 Tip Comb for DW Magnets in a Standard Plate.		

[1] Position on the instrument.

3. Add plant lysate, RNA Binding Beads, and ethanol to the wells of the Sample Plate as indicated in the following table.

Table 9 Sample Plate setup

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Sample Plate[2]	1	Deep Well	Plant lysate	400 µL
			RNA Binding Beads[3]	25 µL
			96–100% Ethanol	400 µL

[1] Position on the instrument.
[2] Add reagents in the order listed in the table.
[3] Resuspend well by vortexing before use.

Bind, wash, treat with DNase I, rebind, and elute the RNA

1. Ensure that the instrument is set up for processing with the deep-well magnetic head and has the standard 96-well heating block installed.
2. Select the **A33784_Plant_RNA_Flex** program on the instrument.
3. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 8 and Table 9).
4. When prompted by the instrument (approximately 29 minutes after initial start):
   a. Remove the DNase I plate from the instrument.

b. Add the following reagents to each sample well to rebind the RNA.
   - 150 µL of Rebinding Buffer
   - 400 µL of 96-100% ethanol

5. Load the DNase I plate back onto the instrument, and press **Start**.

6. At the end of the run (approximately 58 minutes after initial start), remove the Elution Plate immediately from the instrument and seal the wells immediately with an adhesive film.

   (Optional) Eluates can be transferred to a storage plate after collection.

   Note: The plate may be discolored from a remaining bead haze. This minimal bead residue does not interfere with downstream applications.

Store the purified RNA on ice for immediate use. Alternatively, store the purified RNA at –20°C or –80°C for long-term storage.

Purify RNA using the KingFisher™ Duo Prime Magnetic Particle Processor

Set up the processing plate and the Elution Strip

Ensure that ethanol has been added to the Wash Solution Concentrates (see "Before first use of the kit" on page 11).

1. Prepare the DNase I Master Mix for the number of samples to be processed according to the following table.

   IMPORTANT! For best results, prepare the DNase I Master Mix just prior to use to avoid extended periods of time at room temperature.

Component	Volume per sample
2X DNase I Buffer	100 µL
DNase I Solution	4 µL
Manganese Chloride Solution	20 µL
Nuclease-Free Water	76 µL
Total DNase I Master Mix	**200 µL**

2. Add processing reagents to the wells of a KingFisher™ 96-deep well plate as indicated in the following table.

Table 10 Processing plate setup

Row ID	Plate row	Reagent	Volume per well
DNase I	A	DNase I Master Mix	200 µL
Tip Comb	B	Place a KingFisher™ Duo 12-Tip Comb in Row B.	
Sample[1]	C	Plant lysate	400 µL
		RNA Binding Beads[2]	25 µL
		96–100% Ethanol	400 µL
Wash 1_1	D	Wash Solution 1	700 µL
Wash 1_2	E	Wash Solution 1	700 µL
Wash 2_1	F	Wash Solution 2	700 µL
Wash 2_2	G	Wash Solution 2	700 µL
	H	Empty	

[1] Prepare the Sample Row last. Add reagents in the order listed in the table.
[2] Resuspend well by vortexing before use.

3. Prepare the Elution Strip as indicated in the following table.

Table 11 Elution Strip setup

Consumable	Reagent	Volume per well
KingFisher™ Duo Elution Strip	Nuclease-Free Water	100 µL

Bind, wash, treat with DNase I, rebind, and elute the RNA

1. Ensure that the instrument is set up for processing with the 12–pin magnetic head and has the 12–well heating block installed.
2. Select the **A33784_Plant_RNA_Duo** program on the instrument.
3. Start the run and load the prepared processing plate and Elution Strip in their positions when prompted by the instrument (see Table 10 and Table 11).

   Note: Ensure that the Elution Strip is placed in the correct direction into the elution block by ensuring that the perforated end is facing you.

4. When prompted by the instrument (approximately 30 minutes after initial start):
   a. Remove the Processing plate from the instrument.
   b. Add the following reagents to each sample well in Row A to rebind the RNA.
      - 150 µL of Rebinding Buffer
      - 400 µL of 96-100% ethanol
5. Load the Processing plate back onto the instrument, and press **OK**.

6. At the end of the run (approximately 59 minutes after initial start), remove the Elution Strip immediately from the instrument and transfer the eluate to a new tube or plate.

   If using a plate, seal the wells immediately with an adhesive film after having transferred the eluate.

   Note: The Elution Strip may be discolored from a remaining bead haze. This minimal bead residue does not interfere with downstream applications.

Store the purified RNA on ice for immediate use. Alternatively, store the purified RNA at –20°C or –80°C for long-term storage.

Purify RNA manually

Manual purification is performed in 1.5–mL non-stick microcentrifuge tubes.

Ensure that ethanol has been added to the Wash Solution Concentrates (see "Before first use of the kit" on page 11).

Bind the RNA to the beads

1. Add 25 µL of RNA Binding Beads to 400 µL of the plant lysate.

   Note: Resuspend RNA Binding Beads well by vortexing before use.

2. Add 400 µL of 96–100% ethanol, then mix by vortexing for 10 seconds on high setting.

3. Centrifuge briefly to collect the liquid at the bottom of the tube.

4. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are collected against the magnet.

5. Without removing the sample from the magnetic stand, carefully pipette and discard the supernatant.

   Ensure that you remove all the supernatant.

6. Remove the sample from the magnetic stand.

Wash the RNA on the beads

1. Add 700 µL of Wash Solution 1 to the beads, then mix by vortexing for 10 seconds on high setting.

   Note: Beads may clump together, making a uniform suspension difficult to obtain. However, it has no effect on the final yield.

2. Centrifuge briefly to collect the liquid at the bottom of the tube.

3. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are collected against the magnet.

4. Without removing the sample from the magnetic stand, carefully pipet and discard the supernatant.

   Ensure that you remove all the supernatant.

Treat the RNA with DNase I and rebind to the beads

1. Incubate the sample with the lid open for 5 minutes on the magnetic stand to eliminate the remaining ethanol.
2. During the incubation, prepare the DNase I Master Mix for the number of samples to be processed according to the following table.

   IMPORTANT! For best results, prepare the DNase I Master Mix just prior to use to avoid extended periods of time at room temperature.

Component	Volume per sample
2X DNase I Buffer	100 µL
DNase I Solution	4 µL
Manganese Chloride Solution	20 µL
Nuclease-Free Water	76 µL
Total DNase I Master Mix	**200 µL**

3. At the end of the incubation, remove the sample from the magnetic stand, then add 200 µL of DNase I Master Mix to the beads, then tap gently to mix.

   Note: A bead residue may discolor the inside walls of the tube. However, it has no effect on the final yield and quality.
4. Incubate in a thermomixer for 15 minutes at 37°C and 350 rpm.
5. Centrifuge briefly to collect the liquid at the bottom of the tube.
6. Add the following reagents to each sample to rebind the RNA.
   - 150 µL of Rebinding Buffer
   - 400 µL of 96-100% ethanol
7. Mix thoroughly by vortexing for 10 seconds on high setting.
8. Centrifuge briefly to collect the liquid at the bottom of the tube.
9. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are collected against the magnet.
10. Without removing the sample from the magnetic stand, carefully pipet and discard the supernatant.

    Ensure that you remove all the supernatant.
11. Remove the sample from the magnetic stand.

Wash the RNA on the beads after DNase I treatment and rebinding

1. Add 700 µL of Wash Solution 1 to the beads, then mix by vortexing for 10 seconds on high setting.
2. Centrifuge briefly to collect the liquid at the bottom of the tube.
3. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are collected against the magnet.

4. Without removing the sample from the magnetic stand, carefully pipet and discard the supernatant.

   Ensure that you remove all the supernatant.

5. Remove the sample from the magnetic stand.

6. Repeat step 1 through step 5 **twice** with 700 µL of **Wash Solution 2**.

7. Incubate the sample with the lid open for 5 minutes on the magnetic stand to eliminate the remaining ethanol.

Elute the RNA

1. Remove the sample from the magnetic stand.

2. Add 100 µL of Nuclease-Free Water to the beads.

   Note: The volume of Nuclease-Free Water can be reduced to 50 µL if higher RNA concentration is needed or increased to 200 µL if lower RNA concentration is needed.

3. Mix thoroughly by vortexing for 10 seconds on high setting.

4. Centrifuge briefly to collect the liquid at the bottom of the tube.

5. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are collected against the magnet.

6. Without removing the sample from the magnetic stand, carefully transfer the RNA-containing supernatant to a clean tube or plate.

   Note: If using a plate, seal the wells immediately with an adhesive film after transferring the eluate.

   Note: A bead residue may discolor the inside walls of the tube. This minimal bead residue does not interfere with downstream applications.

Store the purified RNA on ice for immediate use. Alternatively, store the purified RNA at –20°C or –80°C for long-term storage.

A

Troubleshooting

Observation	Possible cause	Recommended action
The yield of purified RNA is low	The sample was not stored properly, causing RNA degradation.	Use fresher samples.
		Ensure that samples are processed immediately after collection or removal from storage. If immediate processing is not possible, flash-freeze the samples in liquid nitrogen, then store at –80°C.
	The sample was not sufficiently homogenized.	To adequately disrupt the cell wall and therefore release the RNA, it is important to homogenize the sample thoroughly. Efficient homogenization and lysis of the plant cells increases the RNA yield.
	Ethanol was not added to the lysate.	Ensure that 400 µL of ethanol was added to the lysate to bind the RNA to the RNA Binding Beads.
	The RNA Binding Beads were not completely resuspended.	Resuspend the RNA Binding Beads by vortexing before each use.
	Ethanol was not added to the Wash Solution Concentrates.	Prepare Wash Solution 1 and Wash Solution 2 Concentrates before use as indicated in "Before first use of the kit" on page 11.
	The rebinding step was not performed.	Ensure that Rebinding Buffer and ethanol are added after DNase I treatment.
	RNA Binding Beads were lost during purification.	Use sufficient RNA Binding Beads capture time and be careful not to discard the RNA Binding Beads during the procedure. To prevent aspiration of RNA Binding Beads during the manual purification procedure, aspirate supernatant slowly and keep pipet tip openings away from the captured RNA Binding Beads when aspirating supernatant.
	For manual purification, RNA was adsorbed to the walls of the microcentrifuge tubes.	Use only non-stick microcentrifuge tubes.

Observation	Possible cause	Recommended action
The purified RNA is degraded	The sample was contaminated by RNase.	To avoid RNase contamination, follow the recommended guidelines (see "General procedural guidelines" on page 10).
	The sample was frozen and thawed repeatedly.	Avoid repeated freeze/thaw cycles of the samples.
		Use a new sample for RNA isolation.
		Perform RNA isolation from fresh samples when possible.
	The sample was not stored properly, causing RNA degradation.	Ensure that samples are processed immediately after collection or removal from storage. If immediate processing is not possible, flash-freeze the samples in liquid nitrogen, then store at –80°C.
	The RNA was degraded during sample homogenization.	When homogenizing by mortar and pestle, transfer the ground tissue to the Lysis Buffer quickly as possible to avoid thawing of the sample. All ground material must be thoroughly mixed with the Lysis Buffer. RNA degradation can occur in particles that are left to dry on the walls of the tube.
	The lysis buffer was not supplemented with DTT.	Ensure that 2M DTT solution has been added to the Lysis Buffer before RNA purification procedure as indicated in "Before each use of the kit" on page 11.
	The purified RNA was not stored properly.	Place purified RNA on ice for immediate use in downstream applications or store at -20°C for later use. For prolonged storage (> 1 month), we recommend freezing samples at -80°C.
Downstream enzymatic reactions are inhibited	The purified RNA contains residual salt.	Before the elution step, ensure that the RNA Binding Beads are first washed with Wash Solution 1, then twice with Wash Solution 2.
	The purified RNA contains residual ethanol.	Dry the RNA Binding Beads for 5 minutes at room temperature before the elution step.
	Inhibitors from the sample co-purified with the RNA.	Recalcitrant plants may carry high levels of secondary metabolites such as polyphenolic compounds and polysaccharides. PVP40 or NaCl may be supplemented in the Lysis Buffer with DTT solution to minimize inhibitor carryover (see "Before each use of the kit" on page 11).
		Dilute the DNA sample 10–100 times before the PCR.
		Use enzymes that are designed and optimized to be effective in harsh environments, and work in presence of inhibitors.
		Add Bovine Serum Albumen (BSA) to the PCR.

Observation	Possible cause	Recommended action
The RNA is contaminated by DNA	The DNase I treatment was inefficient.	Ensure that the temperature of DNase I digestion is 37°C.
		Prepare DNase I Master Mix just before use according to protocol.
	Starting amount of plant sample was too high for an effective DNase I treatment.	Reduce the sample input (see "Disrupt the tissue" on page 12 for guidance).
RNA Binding Beads are present in the eluate	Loose beads were inadvertently transferred with the eluate.	Carryover of RNA Binding Beads in the eluted RNA does not affect downstream applications. Centrifuge the eluate at full speed for 1 minute or recapture the beads on the magnetic stand, then transfer the eluate to a new tube or plate.

B

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, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's 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 necessary) 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.

Biological hazard safety

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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.