

Plant/Fungi RNA Purification Kit

Product Insert

Catalog Number E4913

Norgen's Plant/Fungi RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from a wide range of plant and filamentous fungal species. Total RNA can be purified from fresh or frozen plant tissues, plant cells or filamentous fungi samples using this kit. All sizes of RNA are purified, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components, such as proteins, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first homogenizing the cells or tissue with liquid nitrogen (please see the flow chart on page 4). Lysis Buffer C is then added to the tissue or cell powder and is mixed by vortexing. The Lysis Buffer C contains detergents, as well as large amounts of a chaotropic denaturant that will rapidly inactivate RNases and proteases that are present. The lysate is then incubated at 55°C for 5 minutes and spun through the provided Mini Filter Column in order to remove any debris. The lysate is then spun in a microcentrifuge in order to pellet and remove any debris. Ethanol is then added to the clarified lysate, and the solution is loaded onto a spin-column. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA will bind to the column while most of the DNA and proteins are removed in the flowthrough. The bound RNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Kit Specifications

Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Plant Tissues Plant Cells Fungi	50 mg 1 × 10 ⁶ cells 50 mg (wet weight)
Average Yields* 50 mg Tomato Leaves 50 mg Tobacco Leaves 50 mg Plum Leaves 50 mg Grape Leaves 50 mg Peach Leaves	60 µg 60 µg 32 µg 35 µg 30 µg
Time to Complete 10 Purifications	30 minutes

* average yields will vary depending on species, growth conditions used and developmental stage.

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

Kit Components

Component	Amount for 50 samples
Lysis Buffer C	60 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Filter Columns	50
Spin Columns	50
Collection Tubes	100
Elution tubes (1.7 mL)	50
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Advantages

- Fast and easy processing using a rapid spin-column format
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of plant and fungal species
- High yields of total RNA

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Plant/Fungi Total RNA Purification Kit:

- Benchtop microcentrifuge
- 96-100 % ethanol
- Liquid nitrogen
- β -mercaptoethanol (Optional)
- RNase-free DNase I (Optional)

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

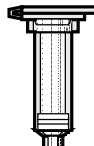
Flow Chart

Procedure for Purifying Total RNA using Norgen's Plant/Fungi Total RNA Purification Kit

Macerate cells or tissue in a mortar using liquid nitrogen.
Add Lysis Buffer C.



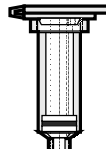
Incubate at 55°C.



Transfer to Filter Column



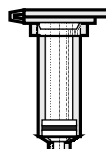
Add Ethanol



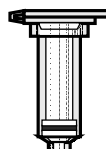
Bind to Spin Column



Wash three times with
Wash Solution A



Elute RNA with
Elution Solution A



Purified Total Plant/Fungi RNA

Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$\text{RPM} = \sqrt{\frac{\text{RCF}}{(1.118 \times 10^{-5}) (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at **14,000 x g** (~ **14,000 RPM**) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 – 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Optional:** Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Buffer C** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Both fresh or frozen samples may be used for this procedure. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen samples to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- It is recommended that no more than 50 mg of fungi, 50 mg of plant tissue or 5 x 10⁶ plant cells be used for this procedure in order to prevent clogging of the column. However, in some cases it may be possible to increase the amount of plant material processed up to 100 mg or 5 x 10⁷ cells, depending on the RNA content of the plant.
- This kit is provided with 2 separate columns. When columns are removed from the labeled bags they are supplied in they can easily be identified as follows:
 - **Filter Columns** – contains a clear plastic O-ring
 - **Spin Columns** – contains a grey plastic O-ring

1. Lysate Preparation

- a. Transfer ≤ 50 mg of plant tissue or 5 x 10⁶ plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle with the liquid nitrogen.

Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.

- b. Transfer the powder to a 2 mL centrifuge tube (not provided) and add 600 µL of **Lysis Buffer C**. Vortex vigorously.
- c. Incubate at 55 °C for 5 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.

- d. Assemble a **Filter Column (clear O-ring)** with one of the provided collection tubes. Pipette the lysate into Filter Column and spin for 2 minutes at 14,000 x g (~14,000 RPM).
- e. Transfer only the clear supernatant from the flow-through into a RNAase-free microcentrifuge tube (not provided) using a pipette.

Note: Ensure that only the clear supernatant is transferred, avoiding any of the debris at the bottom of the collection tube.

- f. Add an equal volume of 96-100 % ethanol (provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2.**

2. Binding to Column

- a. Assemble a **Spin Column (grey O-ring)** with one of the provided collection tubes.
- b. Apply up to 600 µL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with the collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x g (~14,000 RPM)**.

- c. Depending on your lysate volume, repeat step **2b** if necessary.

3. DNase Treatment (Optional)

Norgen's Plant/Fungi Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Norgen Product # 25710) be used for this step. This step should be performed at this point in the protocol

4. Column Wash

- a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Repeat steps **4a** and **4b** to wash column a second time.
- d. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute.
- e. Discard the flowthrough and reassemble the spin column with its collection tube.
- f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50 µL of **Elution Solution A** to the column.
- c. Centrifuge for 2 minutes at **200 x g (~2,000 RPM)**, followed by a 2 minute spin at **14,000 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **14,000 x g (~14,000 RPM)** for 1 additional minute.

- d. **(Optional):** An additional elution may be performed if desired by repeating steps **5b** and **5c**. The total yield can be improved by an additional 20-30% when this second elution is performed.

6. Storage of RNA

The purified RNA sample may be stored at -20°C for a few days. It is recommended that samples be placed at -70°C for long term storage.

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Plant/Fungi Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

- a. For every on-column reaction to be performed, prepare a mix of 15 μL of **DNase I** and 100 μL of **Enzyme Incubation Buffer** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

Note: If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/ μL RNase-free DNase I solution according to the manufacturer's instructions. A 100 μL aliquot is required for each column to be treated.

- b. Perform the Total RNA Isolation Procedure up to and including "**Binding to Column**" (Steps 1 and 2).
- c. Apply 400 μL of **Wash Solution A** to the column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.
- d. Apply 100 μL of the RNase-free DNase I solution prepared in Step a to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.

Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at 14, 000 x g (~14 000 RPM) for an additional minute.

- e. After the centrifugation in Step d, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure Step e is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

- f. Incubate the column assembly at 25 - 30 $^{\circ}\text{C}$ for 15 minutes.
- g. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 4c).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative Elution Solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
Clogged Column	Maximum number of cells or amount of tissue exceeds kit specifications	The optimal input is 50 mg of plant tissue or filamentous fungi, or 5×10^6 plant cells. However, for some species, up to 100 mg of tissue may be processed depending on the RNA content of the sample.
	Too much cell debris in the lysate supernatant	Ensure that most cell debris is removed in Step 1c .
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog.
RNA does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

Related Products	Product #
Plant RNA/DNA Purification Kit	E4788

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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