

Plant RNA/DNA Purification Kit

Product # 24400

Product Insert

Norgen's Plant RNA/DNA Purification Kit provides a rapid method for the isolation and purification of total RNA and DNA simultaneously from a single sample of plants. The total RNA and DNA (including genomic DNA) are both column purified in under 30 minutes using a single column. It is often necessary to isolate total RNA and genomic DNA from a single plant sample, such as for studies of gene expression, mutant or transgenic plant characterization, and host plant-pathogen characterization. Traditionally the RNA and DNA would be isolated from different aliquots of the same sample, however this novel technology will allow for their simultaneous isolation from the same sample. This will not only save time, but will also be of a great benefit when isolating RNA and DNA from precious, difficult to obtain or very small samples. Furthermore, gene expression analysis will be more reliable since the RNA and DNA are derived from the same sample, therefore eliminating inconsistent results.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution (please see the flow chart on page 4). The Lysis Solution contains detergents, as well as large amounts of a chaotropic denaturant that will rapidly inactivate RNases and proteases that are present. A heat treatment is performed to ensure complete lysis. Ethanol is then added to the 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 and DNA will bind to the column while the proteins are removed in the flowthrough. Next, an optional step can be carried out in which the genomic DNA can be digested allowing for a more pure RNA sample to be isolated. The bound nucleic acid is then washed three times with the provided Nucleic Acid Wash Solution in order to remove any impurities, and the purified RNA and/or DNA is eluted with the Nucleic Acid Elution Buffer.

The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). 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. The genomic DNA is of the highest quality, and can be used in PCR reactions, sequencing, Southern blotting and SNP analysis.

Specifications

Kit Specifications	
Column Binding Capacity	50 µg for RNA 15 µg for genomic DNA
Maximum Column Loading Volume	600 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Plant Tissues Plant Cells	100 mg 5 x 10 ⁶
Time to Complete 10 Purifications	30 minutes
Average Yields* Peach Leaves (100 mg)	40 µg RNA, 5 µg gDNA

* average yields will vary depending upon a number of factors including species, growth conditions used and developmental stage.

Kit Components

Component	Product # 24400 (50 samples)
Lysis Solution	40 mL
Nucleic Acid Wash Solution	22 mL
Nucleic Acid Elution Buffer	20 mL
Enzyme Incubation Buffer	6 mL
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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Advantages

- Fast and easy processing using rapid spin-column format
- All columns for total RNA and genomic DNA purification provided
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality genomic DNA and total RNA

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.

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 DNA/RNA Purification Kit:

- Benchtop microcentrifuge
- β -mercaptoethanol
- 96 - 100 % ethanol
- 70% ethanol
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer or bead mills
- Water Bath or Incubator heated to 65°C
- RNase-free DNase I (Optional)
- RNase A (Optional)
- Liquid nitrogen (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 Genomic DNA and Total RNA using Norgen's DNA/RNA Purification Kit

Lyse cells or tissue using **Lysis Solution**



Add Ethanol



Bind to column

SPIN



Wash three times
with Wash Solution

SPIN



Elute DNA and RNA
with Elution Buffer

SPIN



Purified Total RNA and Genomic DNA

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 **Nucleic Acid Wash Solution** by adding 50 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Nucleic Acid Wash Solution**. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Solution** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Pre-heat a water bath or an incubator to 65°C
- The optimal input of plant tissue is 50 mg or 5 x 10⁶ plant cells. However, for most species, up to 100 mg of tissue may be processed.
- Both fresh and frozen plant samples can be used for this protocol. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen tissues 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 important to work quickly when purifying RNA.

1. Lysate Preparation

- a. Transfer 50 mg of plant tissue into an appropriate cell disruption vessel that contains an appropriate amount of liquid nitrogen to cover the sample. Homogenize or grind the sample into a fine powder in liquid nitrogen.

Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen. For fresh, non-frozen tissue, homogenization could also be performed without the use of liquid nitrogen. Place the tissue sample in the cell disruption vessel and proceed directly to Step **1c**.

- b. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

- c. Add 600 μL of **Lysis Solution** (with β -mercaptoethanol) to the tissue sample and continue to homogenize or grind to completion.
- d. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
- e. Incubate the lysate at 65°C for 10 minutes. Mix occasionally by inverting the tube a few times.
- f. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.

Note: Ensure the supernatant is free of cell debris.

- g. Add an equal volume of 70% ethanol (provided by the user, 100 μL of ethanol is added to every 100 μL of lysate). Vortex to mix. **Proceed to Step 2.**

2. Binding Nucleic Acids to Column

- a. Assemble a column 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. 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.

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

3. DNase Treatment (Optional)

This optional step is carried out if genomic DNA-free RNA is required.

- a. Apply 400 μL of **Nucleic Acid Wash Solution** to the column and centrifuge for 2 minutes. Discard the flowthrough.

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. Apply 100 μL of **Enzyme Incubation Buffer** containing 25 units of RNase-free DNase I to the column.

Note: At this point, genomic DNA can be isolated instead of the total RNA. If you wish to isolate RNA-free genomic DNA, apply 100 μL of **Enzyme Incubation Buffer** containing 10 units of RNase A (user provided) to the column and proceed as written below.

- c. Centrifuge for 1 minute at 14,000 $\times g$ (~14,000 RPM). Ensure that the entire DNase I solution passes through the column. Repeat the step if needed.
- d. After centrifugation, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure Step 3d is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA.

- e. Incubate the whole unit at room temperature for 15 minutes.
- f. Proceed to Step **4c** (2nd Column Wash) without further centrifugation.

4. Column Wash

- a. Apply 400 μ L of **Nucleic Acid Wash Solution** 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 column with the collection tube.
- c. Repeat steps **4a** and **4b** to wash column a second time.
- d. Wash column a third time by adding another 400 μ L of **Wash Solution** 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. Nucleic Acid Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 150 μ L of **Nucleic Acid Elution Buffer** to the column.

Note: If only RNA is being isolated, reduce the volume of **Nucleic Acid Elution Buffer** to 100 μ L.

- c. Centrifuge for 2 minutes at **200 x g (~2,000 RPM)**, followed by a 1 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.

Note: For maximum nucleic acid recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

6. Storage of DNA and RNA

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

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Solution was used for the amount of cells or tissue. Ensure that the lysate was incubated at 65°C for 10 minutes. Incubate the lysis solution for an extra 5 minutes to assist in lysis
	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 buffer was used	It is recommended that the Nucleic Acid Elution Buffer 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 Nucleic Acid Wash Solution	Ensure that 50 mL of 95 - 100% ethanol is added to the supplied Nucleic Acid Wash Solution 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.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to “ <i>Working with RNA</i> ” at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to cell disruption in order to ensure that the integrity of the RNA is not compromised.
	Tissue samples were frozen improperly	Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage.

Problem	Possible Cause	Solution and Explanation
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue. Ensure that the lysate was incubated at 65°C for 10 minutes. Incubate the lysis solution for an extra 5 minutes to assist in lysis.
	Maximum number of cells or amount of tissue exceeds kit specifications	The optimal input of plant tissue is 50 mg or 5 x 10 ⁶ plant cells. However, for most species, up to 100 mg of tissue may be processed
	Too much cell debris in the lysate supernatant	Ensure that most cell debris is removed in Step 1f.
	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 Nucleic Acid Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed three times with Nucleic Acid Wash Solution. 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.
Yield of Genomic DNA is Low	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Solution was used for the amount of cells or tissue. Ensure that the lysate was incubated at 65°C for 10 minutes. Incubate the lysis solution for an extra 5 minutes to assist in lysis.
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 x g for 1 minute is performed following the 2 minute centrifugation at 200 x g. Also, ensure that the entire volume of Nucleic Acid Elution Buffer passed through and is eluted from the column.
Genomic DNA is Sheared	Sample is old	Ensure that the sample is not too old, as old samples often yield only degraded DNA
	Sample repeatedly frozen and thawed	Samples should not be repeatedly frozen and thawed, as this tends to increase the likelihood of isolating degraded DNA.

Related Products	Product #
RNA/DNA/Protein Purification Kit	23500
RNA/Protein Purification Kit	23000
1kb RNA Ladder	15003
UltraRanger 1kb DNA Ladder	12100

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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