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Plant RNA/DNA Purification Kit — Product Insert

Product # 24400

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) and 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 Buffer M (please see the flow chart on page 4). The Lysis Buffer M contains detergents, as well as large amounts of a chaotropic denaturant that will rapidly inactivate any RNases that are present. A heat treatment is performed to ensure complete lysis. Next, Binding Buffer I is then added to the lysate followed by a short incubation on ice. The lysate is then spun through the provided Filter Column in order to remove any debris. Ethanol is then added to the clean 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 Wash Solution A in order to remove any impurities, and the purified RNA and/or DNA is eluted with the Elution Buffer E.

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.

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

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 μg for RNA 15 μg for genomic DNA
Maximum Column Loading Volume	650 μL
Average Yields (from 100 mg)* Peach Leaves Raspberry Leaves Strawberry Leaves Grape Leaves	 40 μg RNA, 5 μg gDNA 12 μg RNA, 3 μg gDNA 15 μg RNA, 4 μg gDNA 7 μg RNA, 3 μg gDNA
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Plant Tissues Plant Cells	 100 mg 5×10^6
Time to Complete 10 Purifications	30 minutes

** 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 Buffer M	40 mL
Binding Buffer I	7 mL
Wash Solution A	38 mL
Elution Buffer E	20 mL
Enzyme Incubation Buffer B	6 mL
Filter Columns	50
Spin Columns	50
Collection Tubes	100
Elution tubes (1.7 mL)	50
Product Insert	1

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 Plant RNA/DNA Purification Kit:

- Benchtop microcentrifuge
- 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
- β-mercaptoethanol (Optional)
- RNase-free DNase I (Optional)
- RNase A (Optional)
- Liquid nitrogen

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

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:

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

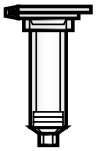
where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Flow Chart

Procedure for Purifying Genomic DNA and Total RNA using Norgen's Plant RNA/DNA Purification Kit

Grind plant or fungi using liquid nitrogen**.**
Add Lysis Buffer M.

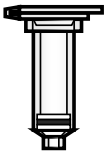
Incubate at 65°C.
Add Binding Buffer I.
Incubate on ice.



Transfer to Filter Column

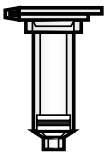
SPIN

Add Ethanol



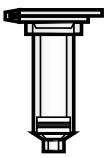
Bind to Spin Column

SPIN



Wash three times with
Wash Solution A

SPIN



Elute DNA and RNA
with Elution Buffer E

SPIN

Purified Total RNA and Genomic DNA

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):** For larger plant samples, or samples with high starch or polysaccharide content, we recommend the use of β-mercaptoethanol during lysis. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Buffer M** 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 10^8 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.
- 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. Place ≤100 mg of plant tissue or wet fungi into a mortar that contains liquid Nitrogen and grind into a powder. Transfer the plant or fungi powder to a DNase-free 1.7 mL microcentrifuge tube (not provided) and add 600 µL of **Lysis Buffer M.**

   Alternatively, other homogenization methods can be used with this procedure, including a bead system. If an alternative method is used, add 600 µL of **Lysis Buffer M** to the sample immediately after homogenization and vortex for 20 seconds to mix.

b. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (provided by user).
c. Incubate the lysate at 65°C for 10 minutes. Mix occasionally by inverting the tube a few times.
d. Add 100 µL of **Binding Buffer I**, mix thoroughly and incubate for 5 minutes on ice.
e. Assemble a **Filter Column (clear O-ring)** with one of the provided collection tubes.
f. Pipette the lysate into the Filter Column and spin for 2 minutes at 14,000 x g (~14,000 RPM).
g. Transfer only the clear supernatant from the flow-through into a DNAase-free microcentrifuge tube (not provided) using a pipette.
h. Add an equal volume of 70% 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 Nucleic Acids 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)

This optional step is carried out if genomic DNA-free RNA is required. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

a. Apply 400 μL of **Wash Solution A** 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 B** containing 15 μL of Norgen's RNase-Free DNase I (Product #25710) to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). If using an alternative DNAse I, apply 100 μL of **Enzyme Incubation Buffer B** containing 25 units of DNase I to the column and centrifuge for 1 minute.

Note: Ensure that the entire DNase I solution passes through the column. Repeat the step if needed. 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 B** containing 10 units of RNase A (user provided) to the column and proceed as written below.

c. After the centrifugation in Step b, pipette the flowthrough that is present in the collection tube back onto the top of the column.

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

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

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 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 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. Nucleic Acid Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 75 µL of **Elution Buffer E** to the column.

Note: If only RNA is being isolated, reduce the volume of **Elution Buffer E** to 100 µL.

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.

Related Products	**Product #**
Plant/Fungi DNA Isolation kit	26200
Plant/Fungi RNA Purification kit	25800
RNA/DNA/Protein Purification Kit	24000
RNA/Protein Purification Kit	24100
RNase-Free DNase I Kit	25710
1kb RNA Ladder	15003
UltraRanger 1kb DNA Ladder	12100

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA/DNA Recovery	Incomplete lysis of cells or tissue	Ensure that the homogenization step was done correctly with the appropriate amount of Lysis Buffer M for the amount of cells or tissue.
	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 Elution Buffer E supplied with this kit be used for maximum RNA/DNA 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/DNA content in cells or tissues used	Different tissues and cells have different RNA/DNA contents, and thus the expected yield of RNA/DNA will vary greatly from these different sources. Please check literature to determine the expected RNA/DNA 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 "*Working with RNA"* 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 M was used for the amount of cells or tissue. Ensure that the lysate was incubated at 65°C for 10 minutes. Incubate the lysis mixture 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 10^8 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 **1e**.
	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/DNA does not perform well in downstream applications	RNA/DNA 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 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.
Yield of RNA/DNA is Low	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Buffer M was used for the amount of cells or tissue. Ensure that the lysate was incubated at 65°C for 10 minutes. Incubate the lysis mixture for an extra 5 minutes to assist in lysis. Liquid nitrogen may be needed for lysis of challenging plant samples.
	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 E passed through and is eluted from the column.
	Binding Buffer I was not added to the lysate	Ensure that the Binding Buffer I is added to the lysate and that it is incubated on ice for 5 minutes prior to spinning down the lysate
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.

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.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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