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Plant/Fungi Total RNA Purification 96-Well Kit Product Insert

Product # 31900

Norgen's Plant/Fungi Total RNA Purification 96-Well Kit provides a rapid method for the high-throughput isolation of total RNA from plants and fungi. 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, Northern blotting, RNase protection, primer extension, and expression array assays.

Norgen's Purification Technology

Purification is based on 96-well 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 purification could be performed on either a vacuum manifold or using centrifugation. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution (please see the flow chart on page 4). Ethanol is then added to the lysate, and the solution is loaded onto the 96-Well Filter Plate. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the resin in the wells, while the contaminating proteins will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Buffer. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Specifications

Kit Specifications	
Binding Capacity Per Well	50 µg
Maximum Loading Volume Per Well	500 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Fungi Plant Tissues	 40 mg 40 mg
Time to Complete 96 Purifications	30 minutes
Average Yields* 25 mg of Grape leaves 40 mg Tomato Leaves 40 mg Tobacco Leaves 40 mg Peach Leaves	 5-7 µg 20-30 µg 20-30 µg 15-20 µg

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

Advantages

- Fast and easy processing using either a vacuum manifold or centrifugation
- No phenol or chloroform extractions
- Isolate high quality total RNA from challenging plant samples

Kit Components

Component	Product #31900 (192 preps)
Lysis Solution	2 x 40 mL
Wash Solution	2 x 40 mL
Elution Buffer	1 x 20 mL
96-Well Filter Plate	2
Adhesive Tape	4
96-Well Collection Plate	2
96-Well Elution Plate	2
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/Fungi Total RNA Purification 96-Well Kit:

- For ***Vacuum Format***:
  - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
  - Sealing tape or pads

- For ***Centrifuge Format***:
  - Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)

- 95 - 100% ethanol
- β-mercaptoethanol
- Collection/Waste Tray for vacuum manifold or 96-well bottom plate (single or 96-well format) for centrifugation
- Cell Disruption Tool such as mortar and pestle, rotor-stator homogenizer or bead mills

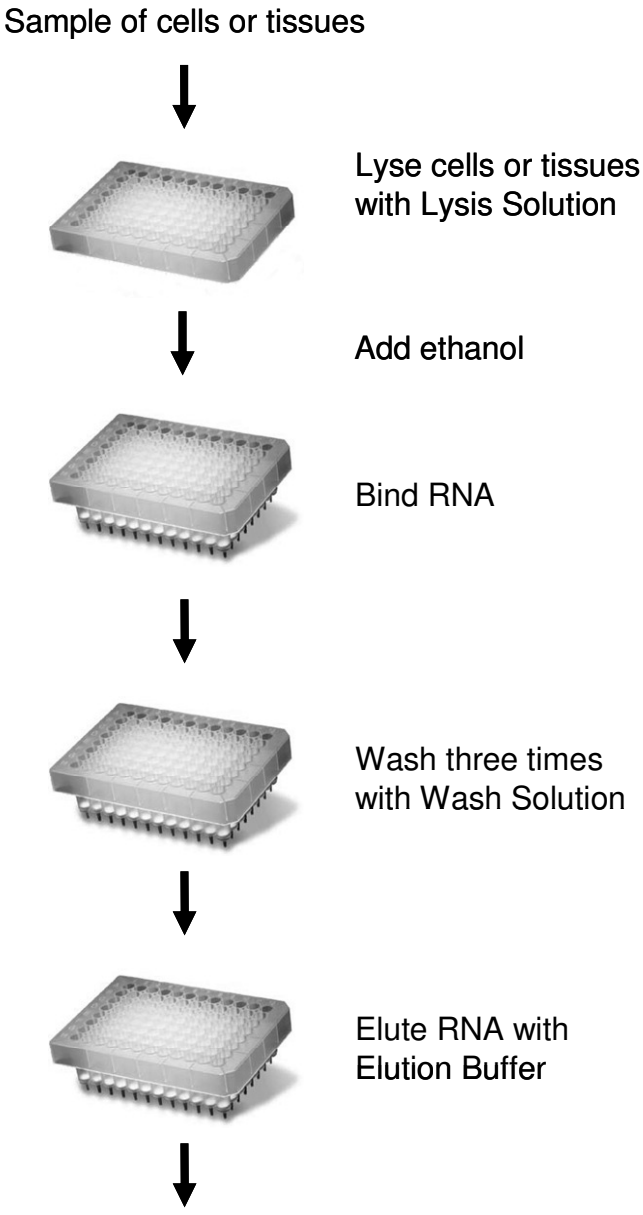
Working with RNA

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

Flowchart

Procedure for Purifying Total RNA using Norgen's Plant/Fungi Total RNA Purification 96-Well Kit



Procedures

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: 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.

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution** by adding 90 mL of 95%-100% ethanol (provided by the user) to each supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 130 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.
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- The maximum recommended input of plant tissue is 40 mg or 5 x 10^6 plant cells per well of the **96-Well Filter Plate**.
- 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 during this procedure.
- The purification of total RNA from the lysate prepared in Step1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section A. For purification using centrifugation, please follow the procedure outlined in Section B

1. Cell Lysate Preparation

a. Obtain a plant or fungi sample and determine the weight or cell number. It is recommended that no more than 40 mg of tissue or 5 x 10^6 plant cells be used per well of the **96-Well Filter Plate**.
b. Transfer the sample to an appropriate vessel for the desired disruption method.
c. Add 400 µL of **Lysis Solution** (ensure that 10 µL of β-mercaptoethanol is added to 1 mL of Lysis Buffer) to each tissue sample.

   Note: Ensure that frozen tissues do not thaw during weighing or prior to the addition of Lysis Solution. For maximum RNA recovery, homogenize frozen tissues to fine powder in liquid nitrogen prior to the addition of **Lysis Solution.**

d. Homogenize the tissues using the appropriate cell disruption tool.

Note: Other homogenization methods, including grinding with liquid nitrogen, can be applied to this procedure. If an alternative method is used, add 400 µL of **Lysis Solution** to the sample immediately after homogenization and vortex for 20 seconds to mix.

e. Transfer all the lysate to RNase free 1.7 mL microtube (provided by the user) and centrifuge at 14,000 x g (~14,000 RPM) for 2 minutes.
f. Using a pipette, transfer the clean lysate into a RNAase-free microcentrifuge tube (not provided).
g. 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.**

Note: The purification of total RNA from the lysate prepared in Step 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section A. For purification using centrifugation, please follow the procedure outlined in Section B

A. Plant/Fungi Total RNA isolation Using Vacuum Manifold

2. Binding RNA to 96-Well Filter Plate

a. Assemble the 96-Well Filter Plate and the vacuum manifold according to manufacturer's recommendations. Apply up to 400 µL of the lysate mixed with the ethanol (from **Step 1**) into each well of the 96-Well Filter Plate.

Note: The provided 96-Well Collection Plate can be used as the collection/waste tray if desired.

b. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application of the mixture to the wells.

c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the vacuum manifold.

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

d. Apply the remainder of the lysate mixed with the ethanol into each well and repeat steps **2b** and **2c**.

Optional Step:

Norgen's Plant/Fungi Total RNA Purification 96-Well Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Plate DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol.

3. RNA Wash

a. Apply 400 µL of **Wash Solution** to each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution has passed through into the collection/waste tray by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a second time.
d. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a third time.
e. Pat the bottom of the 96-Well Filter Plate dry. Reassemble the 96-Well Filter Plate and the vacuum manifold. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
f. Turn off vacuum and ventilate the manifold.

4. RNA Elution

a. Replace the collection/waste tray in the vacuum manifold with the provided 96-Well Elution Plate. Complete the vacuum manifold assembly with the 96-Well Filter Plate.
b. Add 75 µL of **Elution Buffer** to each well of the plate.
c. Apply vacuum for 2 minutes.

5. Storage of RNA

Use the provided adhesive tape to seal the Elution Plate. The purified RNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

B. Plant/Fungi Total RNA Purification Using Centrifugation

2. Binding RNA to 96-Well Filter Plate

a. Place the 96-Well Filter Plate on top of a provided 96-Well Collection Plate
b. Apply up to 300 µL of the lysate mixed with the ethanol (from **Step 1**) into each well of the 96-Well Filter Plate.
c. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application to the wells

c. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the 96-Well Collection Plate and repeat Step B2 b and c with remained lysate mixture.

Note: Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

3. RNA Wash

a. Apply 400 µL of **Wash Solution** to each well of the **96-Well Filter Plate**. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the bottom plate by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the 96-Well Collection Plate.
c. Repeat steps **3a** and **3b** to wash column for a second time.
d. Repeat steps **3a** and **3b** to wash column for a third time.
e. Pat the bottom of the 96-Well Filter Plate dry. Reassemble the 96-Well Filter Plate and the 96-Well Collection Plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 5 minutes in order to completely dry the plate.

4. RNA Elution

a. Stack the 96-Well Filter Plate on top of the 96-Well Elution Plate.
b. Add 75 µL of **Elution Buffer** to each well of the 96-Well Filter Plate.
c. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

5. Storage of RNA

Use the provided adhesive tape to seal the Elution Plate. 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 96-Well 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 an RNase-free DNase I be used.

1. Prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 75 µL aliquot is required for each well to be treated. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 8.0, 10 mM $MgCl_2$ and 3 mM $CaCl_2$, made RNase-free) to give a final concentration of 0.25 Kunitz unit/µL.

2. Perform the procedure up to and including "**Binding RNA to 96-Well Filter Plate**" (Steps 1 and 2 of all protocols)

3. ***For Vacuum Manifold*****:** Apply 400 µL of **Wash Solution** to each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or a pad (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

   For Centrifugation**:** Apply 400 µL of **Wash Solution** to each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

4. Discard the flowthrough. Reassemble the 96-Well Filter Plate with the vacuum manifold or the bottom plate.

5. Apply 75 µL of the RNase-free DNase I solution prepared in Step 1 to each well of the 96-Well Filter Plate.

   For Vacuum Manifold**:** Apply vacuum for 30 seconds.
   For Centrifugation**:** Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 30 seconds.

6. Incubate the assembly at 25 - 30°C for 15 minutes.

7. Without any further centrifugation, proceed directly to "**RNA Wash**" Section 2A, Step 3b for **Vacuum Manifold** procedure or Section 2B, Step 3c for **Centrifugation** procedure.

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.
	Wells of the plate have become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the wells of the plate show clogging below the recommended levels. See also "Clogged Wells in Plate" below.
	An alternative elution solution was used	It is recommended that the 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 wells of the plate.
	Ethanol was not added to the Wash Solution	Ensure that 90 mL of 95% ethanol is added to the supplied 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.
Clogged Wells in Plate	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications
Clogged Wells in Plate	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog.

Problem	Possible Cause	Solution and Explanation
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. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	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 isolation in order to ensure that the integrity of the RNA is not compromised.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Lysis Solution.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the plate is not washed 3 times with Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry vacuum or dry spin under the RNA Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNase I digestion on the RNA sample after elution to remove genomic DNA contamination.

Related Products	Product #
Plant/Fungi Total RNA Purification Kit (50 preps)	25800
Plant/Fungi Total RNA Purification Kit (96 preps)	31300
Total RNA Purification Kit	17200
RNA/Protein Purification Kit	23000
RNA/DNA/Protein Purification Kit	23500
Cytoplasmic & Nuclear RNA Purification Kit	21000
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

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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