NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

# Plant/Fungi Total RNA Purification Kit — Product Insert

**Product # 25800**

Norgen's Plant/Fungi Total 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 procedure is rapid and convenient, as it does not rely on the use of liquid nitrogen in order to homogenize the samples. 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 macerating the cells or tissue in a mortar 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. Alternatively, liquid nitrogen can be used to homogenize the sample. 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 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 | |
|---|---|
| Column Binding Capacity | 50 µg |
| Maximum Column Loading Volume | 600 µL |
| Size of RNA Purified | All sizes, including small RNA (<200 nt) |
| Maximum Amount of Starting Material:<br>Plant Tissues<br>Plant Cells<br>Fungi | <br>50 mg<br>1 × $10^6$ cells<br>50 mg (wet weight) |
| Average Yields*<br>50 mg Tomato Leaves<br>50 mg Tobacco Leaves<br>50 mg Plum Leaves<br>50 mg Grape Leaves<br>50 mg Peach Leaves | <br>60 µg<br>60 µg<br>32 µg<br>35 µg<br>30 µg |
| 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 # 25800 (50 samples) |
|---|---|
| Lysis Solution | 40 mL |
| Wash Solution | 22 mL |
| Elution Buffer | 6 mL |
| Mini Spin Columns | 50 |
| Collection Tubes | 50 |
| Elution tubes (1.7 mL) | 50 |
| Product Insert | 1 |

## Advantages

- Fast and easy processing using a rapid spin-column format
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No liquid nitrogen is required
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of plant and fungal species
- High yields of 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 Plant/Fungi Total RNA Purification Kit:

- Benchtop microcenrifuge
- 96-100 % ethanol
- 70 % ethanol
- β-mercaptoethanol
- RNase-free DNase I (Optional)
- Liquid nitrogen (Optional)

**Working with RNA**

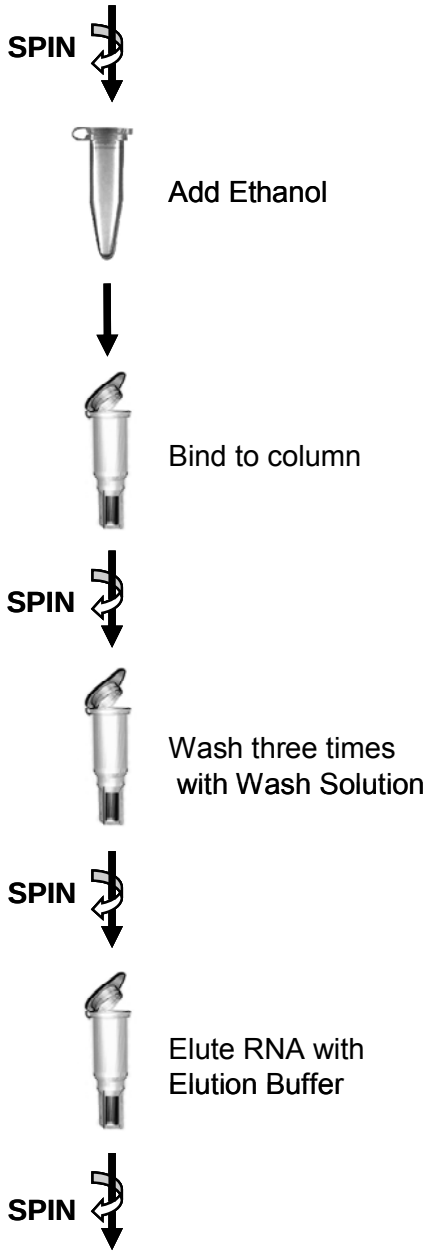
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense 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 Total RNA using Norgen's Plant/Fungi Total RNA Purification Kit

Macerate cells or tissue in a mortar using **Lysis Solution**




**Purified Total 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:

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

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***

- 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** by adding 50 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **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.
- 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.
- While the provided procedure does not rely on the use of liquid nitrogen to homogenize the sample, both fresh and frozen tissues can optionally be processed using other homogenization methods, including grinding with liquid nitrogen. Please refer to the Note in Step 1a.
- It is recommended that no more than 50 mg of fungi, 50 mg of plant tissue or $5 \times 10^6$ 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 $5 \times 10^7$ cells, depending on the RNA content of the plant.

**1. Lysate preparation**

a. Transfer ≤50 mg of plant tissue or $5 \times 10^6$ plant cells into a mortar that contains 600 µL of **Lysis Solution**. Grind the sample using a pestle until the tissue is completely macerated.

**Note:** Other homogenization methods, including grinding with liquid nitrogen, can be applied to this procedure. If an alternative method is used, add 600 µL of **Lysis Solution** to the sample immediately after homogenization and vortex for 20 seconds to mix.

b. Using a pipette, transfer the lysate into an RNAase-free microcentrifuge tube (not provided).
c. 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:** Depending on the plant or fungal species, large amounts of debris may be present in the supernatant. Ensure that only the clear supernatant is transferred, avoiding any of the debris. If necessary, repeat Step **1c** if visible precipitates are still present after the first spin.

d. 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 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 at **14000 × g (~14,000 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.

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

## 3. Column Wash

a. Apply 400 μL of **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 spin column with its collection tube.
c. Repeat steps **3a** and **3b** to wash column a second time.
d. Wash column a third time by adding another 400 μL 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.

**4. RNA Elution**

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 μL of **Elution Buffer** to the column.
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.
d. **(Optional):** An additional elution may be performed if desired by repeating steps **4b** and **4c**. The total yield can be improved by an additional 20-30% when this second elution is performed.

**5. 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.

## 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 buffer 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 column. |
| | Ethanol was not added to the Wash Solution | Ensure that 50 mL of 95 - 100% 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 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 x $10^6$ 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 | Traces of salt from the binding step may remain in the sample if the column is not washed three times with the 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. |

| Related Products | Product # |
|---|---|
| Plant RNA/DNA Purification Kit | 24400 |
| Total RNA Purification Kit | 17200 |
| 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.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362