

Soil Total RNA Purification Kit

Product #27750

Product Insert

Norgen's Soil Total RNA Purification Kit provides a convenient and rapid method to purify total RNA from small amounts of soil samples. All types of soil samples can be processed with this kit, including common soil samples and difficult soil samples with high humic acid content such as compost and manure. The kit removes all traces of humic acid using the provided Bead Tubes and a combination of chemical and physical homogenization and lysis. A simple and rapid spin column procedure is then used to further purify the RNA. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA and small interfering RNA. The protocol does not rely on the use of phenol or chloroform, thereby providing a user friendly procedure and allowing high-throughput analysis on the lab bench. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR and reverse transcription PCR for gene expression analysis.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The process involves first adding the soil sample and Lysis Buffer I to a provided Bead Tube, and the tube is vortexed for 5 minutes in order to efficiently and rapidly homogenize the sample and extract the RNA. The sample is then centrifuged, and the supernatant is transferred to a RNase-free microcentrifuge tube. Binding Buffer E and Solution BX are added sequentially and mixed by inversion, and the lysate is incubated for 5 minutes on ice. The lysate is then spun through a Humic Acid Removal Column to remove all humic acids and the flow through is collected and Lysis Buffer QP and ethanol are added. The solution is then loaded onto a spin-column. Norgen's resin binds RNA in a manner that depends on ionic concentrations. An optional on-column DNase treatment can be performed at this point also to remove any residual DNA. The bound RNA is then washed once with the provided Binding Buffer B and twice with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution A. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Specifications

| Kit Specifications | |
|--|--|
| Suggested Soil Input (Clay, loam, sand, feces, compost) | 500 mg |
| Type of Soil Processed | All types, including common soil, compost and manure |
| Maximum Column Binding Capacity | 50 µg |
| Maximum Column Loading Volume | 650 µL |
| Time to Complete 10 Purifications | 30 minutes |

Advantages

- No phenol or chloroform extractions
- Fast and easy processing (less than 30 minutes for purification)
- Isolate high quality total RNA from a variety of soil samples
- Process all types of soil, including common soil, compost and manure

Kit Components

| Component | Product # 27750 (50 preps) |
|----------------------------|----------------------------|
| Lysis Buffer I | 2 x 20 mL |
| Binding Buffer E | 6 mL |
| Solution BX | 9 mL |
| Lysis Buffer QP | 25 mL |
| Binding Buffer B | 30 mL |
| Wash Solution A | 18 mL |
| Elution Solution A | 6 mL |
| Bead Tubes | 50 |
| Spin Columns | 50 |
| Humic Acid Removal 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. **Solution BX, Lysis Buffer QP and Binding Buffer B** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. 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 Norgen's Soil Total RNA Purification Kit:

- Benchtop microcentrifuge
- RNase-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment
- 96-100% ethanol
- 70 % ethanol (Freshly prepared)

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 Soil Total RNA using Norgen's Soil Total RNA Purification Kit

Add soil sample and Lysis Buffer I to Bead Tube

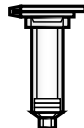


Vortex for 5 minutes.
Centrifuge. Transfer lysate.



Add Solution BX and Binding
Buffer E.
Incubate for 5 minutes on ice.

SPIN

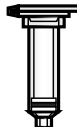


Transfer Lysate to Humic
Acid Removal Column

SPIN

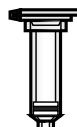


Transfer supernatant. Add
Lysis Buffer QP and
70% ethanol. Vortex



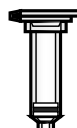
Bind to column

SPIN



Wash once with Binding Buffer B.
Wash two times with Wash Solution A

SPIN



Elute RNA with
Elution Solution A

SPIN



Purified Total RNA

Procedure

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.

Suggested centrifuge speed is based on Sorvall Legend Micro Centrifuges (Thermo Fisher Scientific Inc.)

Notes Prior to Use

- 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 **Wash Solution A** by adding 42 mL of 96 - 100 % ethanol (provided by the user) to each supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 60 mL. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- This kit is provided with 2 separate columns. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:
 - Humic Acid Removal Columns – column has blue and white contents
 - Spin Columns – column has grey and white contents

1. Lysate Preparation

- a. Add 300 mg of soil sample to a Bead Tube and add 700 µL of **Lysis Buffer I**. Vortex briefly to mix soil and Lysis Solution.

Note: In case of a wet soil sample, transfer the sample to a clean 1.7 mL microcentrifuge tube and centrifugation for 30 seconds at **20,000 × g (~14,000 RPM)**. Remove the water carefully using a pipette, and resuspend the soil pellet in 700 µL of **Lysis Buffer I**. Transfer the soil to a Bead Tube using a pipette.
Proceed to Step 1b.

- b. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. OMNI Bead Ruptor 24 Elite). For OMNI Bead Ruptor 24 Elite homogenize for 1 minute at speed 4 (m/s), or 5 minutes using a flat-bed vortexer at maximum speed. Alternatively, optimize the time and speed according to the manufacturer's manual.
- c. Centrifuge the tube for 2 minutes at **20,000 × g (~14,000 RPM)**.
- d. Transfer up to 450 µL of supernatant to an RNAase-free microcentrifuge tube (not provided).
- e. Add 50 µL of **Solution BX** and add 50 µL **Binding Buffer E** sequentially. Mix by inverting the tube a few times.
- f. Incubate for 5 minutes on ice.
- g. Spin the lysate for 1 minute at **20,000 × g (~14,000 RPM)** to pellet any protein and soil particles.

- h. Using a pipette, transfer up to 450 μL of supernatant into a **Humic Acid Removal Column (blue and white contents)** without any contact with the pellet.
- i. Spin the column at 4,000 $\times g$ (~8,000 rpm) for 30 seconds. **Don't discard the flow through that contains RNA.**
- j. Using a pipette, transfer up to 400 μL of supernatant (avoid any contact with the pellet when collecting the supernatant) into a RNAase-free microcentrifuge tube (not provided).
- k. Add 300 μL of Lysis Buffer QP and 700 μL of 70 % ethanol (provided by the user) to the lysate collected above. Vortex to mix. **Proceed to Step 2.**

2. Binding to Column

- a. Assemble a **Spin Column (grey and white contents)** with one of the provided collection tubes.
- b. Apply up to 650 μL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **20,000 $\times g$ (~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 at **20,000 $\times g$ (~14,000 RPM)**.

- c. Repeat step **2b** with the remaining lysate.

Optional Step:

Norgen's Soil 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 (Product # 25710) be used for this step. This step should be performed at this point in the protocol. Otherwise proceed directly to Step 3 below.

3. Column Wash

- a. Apply 500 μL of **Binding Buffer B** to the column and centrifuge for 1 minute at **20,000 $\times g$ (~14,000 RPM)**.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Apply 500 μL of **Wash Solution A** to the column and centrifuge for 1 minute at **20,000 $\times g$ (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
- d. Repeat **3c**.
- e. Spin the column for 2 minutes at **20,000 $\times g$ (~14,000 RPM)** 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 Solution A** to the column.
- c. Centrifuge for 2 minutes at **200 $\times g$ (~1,500 RPM)**, followed by a 1 minute spin at **20,000 $\times g$ (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **20,000 $\times g$ (~14,000 RPM)** for 1 additional minute.

5. Storage of RNA

The purified RNA 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 Soil Total RNA Purification Kit isolates total RNA with minimal amounts of 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.

1. For every on-column reaction to be performed, prepare a mix of $15\ \mu\text{L}$ of **DNase I** and $100\ \mu\text{L}$ of **Enzyme Incubation Buffer A** 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 $100\ \mu\text{L}$ aliquot is required for each column to be treated.

2. Perform Steps 1 and 2 of the protocol (Lysate Preparation and Binding to Column).
3. Apply $500\ \mu\text{L}$ of **Binding Buffer B** to the column and centrifuge for 1 minute at $20,000 \times g$ ($\sim 14,000$ RPM).
4. Discard the flowthrough and reassemble the spin column with its collection tube.
5. Apply $500\ \mu\text{L}$ of **Wash Solution A** to the column and centrifuge for 1 minute at $20,000 \times g$ ($\sim 14,000$ RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
6. Apply $100\ \mu\text{L}$ of the RNase-free **DNase I** solution prepared in Step 1 to the column and centrifuge at $20,000 \times g$ ($\sim 14,000$ RPM) for 1 minute.

Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at $20,000 \times g$ ($\sim 14,000$ RPM) for an additional minute.

7. After the centrifugation in Step 6, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure Step 7 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

8. Incubate the column assembly at $25 - 30^{\circ}\text{C}$ for 15 minutes.
9. Without any further centrifugation, proceed directly to the second wash step with **Wash Solution A** in the "Column Wash" section (Step 3d).

Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|-------------------|--|--|
| Poor RNA Recovery | Sample is not fresh | It is critical to use a freshly collected soil sample. In the case of a frozen soil sample, slowly defrosting at 4°C is recommended to minimize the degradation of RNA. |
| | Homogenization was incomplete | Depending on the type of soil, further vortexing with the flat bed vortex or bead beater equipment may be required. However, it is not recommended to increase the vortex time to longer than 10 minutes at maximum speed (flat-bed vortexer). |
| | Column has become clogged | Do not exceed the recommended input amount of 500mg mg soil. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below. |
| | 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 42 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use. |
| 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. |
| | 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. |
| | DNase used may not be RNase-free | Ensure that the DNase being used for the optional On-Column DNA Removal step RNase-free, in order to prevent possible problems with RNA degradation. |

| Problem | Possible Cause | Solution and Explanation |
|--|--|---|
| RNA does not perform well in downstream applications | RNA was not washed with Binding Buffer B and Wash solution A | Traces of humic acids and salt from the binding step may remain in the sample if the column is not washed with Binding Buffer B and 2 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. |

| Related Products | Product # |
|-----------------------------|------------------|
| RNase-Free DNase I Kit | 25710 |
| Soil DNA Isolation kit | 26500 |
| Soil DNA Isolation Plus Kit | 64000 |
| 1kb RNA Ladder | 15003 |
| HighRanger 1kb DNA Ladder | 11900 |
| 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.

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

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