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

Soil Total RNA Purification Kit - Supplementary Protocol for Isolation of RNA from Wastewater

Product # 27750

Customer-Supplied Reagents

- Centricon® Plus-70 Centrifugal Filter with a cut-off of 10 kDa (Merck Millipore)
- Benchtop microcenrifuge
- RNAse-free microcentrifuge tubes
- 96-100% ethanol
- 70 % ethanol (Freshly prepared)

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. Sample Treatment

a) Concentrate the sample using the Centricon® Plus-70 Centrifugal Filter with a cut-off of 10 kDa (Merck Millipore).
b) Transfer the concentrated sample (approximately 250 µL) from the concentrate collection cup into an RNase-free microcentrifuge tube (provided by the user).

2. Lysate Preparation

a) Add 50 µL of **Solution BX** and add 50 µL **Binding Buffer E** sequentially. Mix by inverting the tube a few times.
b) Incubate for 5 minutes on ice.
c) Spin the lysate for 1 minute at **20,000 × g (~14,000 RPM)** to pellet any protein and soil particles. If the lysate has a clear colour, skip Step **2d**.
d) 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.
e) Spin the column at **4,000 x g (~8,000 rpm)** for 30 seconds. ***Don't discard the flow through that contains RNA.***
f) Using a pipette, transfer up to 400 µL of supernatant (avoid any contact with the pellet when collecting the supernatant) into a RNase-free microcentrifuge tube (not provided).
g) 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 3**.

3. 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 × 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 × g (~14,000 RPM)**.

c) Repeat step 3b with the remaining lysate.

4. Column Wash

a) Apply 500 µL of **Binding Buffer B** to the column and centrifuge for 1 minute at **20,000 × 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 × g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
d) Repeat **4c**.
e) Spin the column for 2 minutes at **20,000 × g (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

5. 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 x g (~1,500 RPM)**, followed by a 1 minute spin at **20,000 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **20,000 x g (~14,000 RPM)** for 1 additional minute.

6. 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.

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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