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

Total RNA Purification Kit - Supplementary Protocol for Isolation of RNA from Norgen's Swab Collection and Total Nucleic Acid Preservation System (Cat. 68800)

Notes Prior to Use

- Bodily fluid of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with saliva.
- Refer to manufacturer's product documentation for specific instructions on saliva collection, preservation and safety information.
- 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.

1. Saliva Collection

a. Collect swab into Norgen's Swab Collection and Total Nucleic Acid Collection and Preservation Device according to the manufacturer's instruction.
b. Refer to manufacturer's instructions for storage and shipment conditions.

2. Lysate Preparation

a. Mix the preserved swab sample by vortexing the tube for 10 seconds.
b. Transfer 250 µL of the preservative to an RNase-free microcentrifuge tube.
c. Add 250 µL of **Buffer RL**. Vortex the tube for a few seconds to mix.
d. Add 250 µL of **96-100% ethanol**. Vortex briefly to mix.

3. Binding RNA to Column

a. Assemble a Spin Column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate with the ethanol (from **Step 2d**) onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).**

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. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Step **3b** and **3c** as necessary.

Optional Step:

Norgen's 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 of the full version manual available on our website 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

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 spin column with its 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. 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* (~2,000 RPM)**, followed by 1 minute at **14,000 x *g* (~14,000 RPM)** Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

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