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Saliva/Swab RNA Purification Kit — Product Insert

Product # 69100

Norgen's Saliva/Swab RNA Purification Kit provides a rapid method for the purification of total RNA from non-preserved saliva and nasal/throat swabs, and from preserved saliva collected on Norgen's Saliva RNA Collection and Preservation Devices (Cat. RU53800) or preserved swabs collected in Norgen's Total Nucleic Acid Preservative Tubes (Cat. 69200). Purification is based on using Norgen's proprietary resin separation matrix. RNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform. The kit allows the purification of total RNA, including viral and bacterial RNA, irrespective of size or GC content. The purified RNA is eluted in an Elution Solution that is compatible with all downstream applications including PCR, qPCR, methylation-sensitive PCR and Southern Blot analysis, microarrays and NGS.

Specifications

Kit Specifications	
Sample Volume Range	250 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Minimum Elution Volume	50 µL
Maximum Elution Volume	100 µL
Time to Complete 10 Purifications	15 - 20 minutes
Average Yield	≥ 1 µg * *Varies from sample to sample

Kit Components

Component	Product # 69100
Lysis Buffer A	30 mL
Solution WN	18 mL
Wash Solution A	18 mL
Elution Solution A	6 mL
Mini Spin Columns	50
Collection Tubes	50
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. This kit is stable for 2 years after the date of shipment.

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 Safety Data Sheets (SDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Body 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 these samples.

Lysis Buffer A and **Solution WN** contains 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

Customer-Supplied Reagents and Equipment
You must have the following in order to use the Saliva/Swab RNA Purification Kit:

For All Protocols

- Benchtop microcentrifuge
- 96 - 100% ethanol
- 1x PBS (pH 7.4)
- β-mercaptoethanol (optional)

For Preserved Saliva Samples

- Norgen's Saliva RNA Collection and Preservation Devices (RU53800)

For Preserved Nasal or Throat Swabs

- Norgen's Total Nucleic Acid Preservative Tubes (Cat. 69200)
- Sterile nylon flocked swabs

For Non-Preserved Nasal or Throat Swabs

- Sterile nylon flocked swabs

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

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 **Solution WN** by adding 24 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Solution WN**. This will give a final volume of 42 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Optional:** The use of β-mercaptoethanol in lysis is highly recommended for nasal and throat swabs. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Buffer A** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, Lysis Buffer A can be used as provided.
- It is important to work quickly during this procedure.

1A. Lysate Preparation from Preserved Saliva Sample

Notes Prior to Use

- Saliva samples must be collected on Norgen's Saliva RNA Collection and Preservation Devices (Cat. RU53800) as per the instructions.

a. Transfer 250 µL preserved saliva sample into a 2 mL tube. Add 1x PBS pH 7.4 to make up the volume to 400 µL.
b. Add 400 µL of **Lysis Buffer A** directly to the previous mix. Mix by vortexing for 10 seconds.
c. Add 400 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

1B. Lysate Preparation from Preserved Nasal or Throat Swabs

Notes Prior to Use

- Nasal or throat swabs must be collected and preserved in Norgen's Total Nucleic Acid Preservative Tubes (Cat. 69200) as per the instructions.

a. Collect nasal or throat swab and place into preservative as per the instructions in Norgen's Total Nucleic Acid Preservation Tubes (Cat. 69200).
b. Transfer 250 µL preserved swab sample in a 2 mL tube. Add 1x PBS pH 7.4 to make up the volume to 400 µL.
c. Add 400 µL of **Lysis Buffer A** directly to the previous mix. Mix by vortexing for 10 seconds.
d. Add 400 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

1C. Lysate Preparation from Non-Preserved Saliva

Notes Prior to Use

- Fresh saliva samples should be used

a. Transfer 250 µL saliva sample in a 2 mL tube. Add 1x PBS pH 7.4 to make up the volume to 400 µL.
b. Add 400 µL of **Lysis Buffer A** directly to the previous mix. Mix by vortexing for 10 seconds.
c. Add 400 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

1D. Lysate Preparation from Non-Preserved Nasal or Throat Swabs

Notes Prior to Use

- Swab samples should be collected using sterile nylon flocked swabs and processed immediately

a. Add 400 µL of **Lysis Buffer A** to an RNase-free microcentrifuge tube (not provided). Mix by vortexing for 10 seconds.
b. Gently brush a sterile, nylon flocked swab inside the nose or mouth of the subject.
c. Using sterile techniques, cut the swab tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the Lysis Buffer A. Close the tube. Vortex gently and incubate for 5 minutes at room temperature.
d. Using a pipette, transfer the lysate into another RNase-free microcentrifuge tube (not provided). Note the volume of the lysate.
e. Add 400 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

Section 2. Total RNA Purification from All Types of Lysate

Note: The remaining steps of the procedure for the purification of total RNA are the same from this point forward for all the different types of lysate.

2. Binding RNA to Column

a. Assemble a column with one of the provided collection tubes
b. Apply up to 600 µL of the lysate with the ethanol (from **Step 1**) onto the column and centrifuge for 1 minute at **20,800 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 **2b** and **2c** as necessary to bind the remaining lysate volume.

Optional Step:

Norgen's Saliva/Swab RNA Purification Kit isolates 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.

3. Column Wash

a. Apply 400 µL of **Solution WN** to the column and centrifuge for 1 minute.

Note: Ensure the entire volume of Solution WN 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. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat steps **3c** and **3d** to wash column a second time with Wash Solution A.
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 Solution A** to the column.
c. Centrifuge for 2 minutes at **420 x *g* (~2,000 RPM)**, followed by 1 minute at **20,800 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 20,800 x g (~14,000 RPM) for 1 additional minute.

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.

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Saliva/Swab RNA Purification 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 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 µL of **DNase I** and 100 µL 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 µL aliquot is required for each column to be treated.

2. Perform the appropriate Saliva/Swab RNA Isolation Procedure for your starting material up to and including "**Binding to Column**" (Steps 1 and 2 of all protocols)

3. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.

4. Apply 100 µL of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.

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

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

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

7. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 3c).

Related Products	Product #
Saliva RNA Collection and Preservation Devices	RU53800
Total Nucleic Acid Preservative Tube	69200

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Saliva/Swab RNA Purification Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. or call one of the NORGEN local distributors (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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