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Saliva/Swab RNA Purification 96-Well Kit — Product Insert

Product # 69300

Norgen's Saliva/Swab RNA Purification 96-Well Kit provides a rapid method for the high-throughput 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 real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays

Kit Components

Component	Product # 69300 (192 preps)
Lysis Buffer A	100 mL
Solution WN	55 mL
Wash Solution A	2 x 38 mL
Elution Solution A	20 mL
96-Well Isolation Plate (Deep Well)	2
96-Well Collection Plate (Deep Well)	2
96-Well Elution Plate (Deep Well)	2
Adhesive Tape	4
Product Insert	1

Specifications

Kit Specifications	
Sample Volume Range	250 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Minimum Elution Volume	75 µL
Maximum Elution Volume	100 µL
Time to Complete 96 Purifications	30 minutes
Average Yield	≥ 1 µg * *Varies from sample to sample

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 96-Well Kit:

For All Protocols
- For ***Vacuum Format***:
  - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
  - Sealing tape or pads
- For ***Centrifuge Format***:
  - Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)
- Collection/Waste Tray for vacuum manifold or 96-well bottom plate (single or 96-well format) for centrifugation. Two 96-Well Collection Plates are provided with the kit.
- 96 - 100% ethanol
- 1x PBS (pH 7.4)
- β-mercaptoethanol (optional)
- 2mL RNase-free microcentrifuge tubes

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

Procedures

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: 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.

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

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Solution WN** by adding 73 mL of 96-100% ethanol (provided by the user) to the supplied bottles containing the concentrated **Solution WN**. This will give a final volume of 128 mL. The labels on the bottles have 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:
  - 90 mL of 96 - 100% ethanol (provided by the user) to each of the bottles containing 38 mL of concentrated **Wash Solution A**. This will give a final volume of 128 mL.
  - 350 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing 148 mL concentrated **Wash Solution A**. This will give a final volume of 498 mL.

  The labels on the bottles have 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 into 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).
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).
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 purification of total RNA from the lysate prepared in Section 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B

A. Total RNA Purification from All Types of Lysate Using Vacuum Manifold

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

2. Binding RNA to 96-Well Isolation Plate (Deep Well)

a. Assemble the 96-Well Isolation Plate (Deep Well) and the vacuum manifold according to manufacturer's recommendations.

Note: The provided 96-Well Collection Plate (Deep Well) can be used as the collection/waste tray if desired.

b. Apply up to 600 µL of the lysate with the ethanol (from **Step 1**) into each well of the 96-Well Isolation Plate (Deep Well). Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application of the mixture to the wells.

c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Isolation Plate (Deep Well) and the vacuum manifold.
d. Repeat Step **2b** and **2c** as necessary to bind the remaining lysate volume.

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

3. RNA Wash

a. Apply 400 µL of **Solution WN** to each well of the 96-Well Isolation Plate (Deep Well). Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire **Solution WN** has passed through into the collection/waste tray by inspecting the 96-Well Isolation Plate (Deep Well). If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Isolation Plate (Deep Well) and the vacuum manifold.
d. Apply 400 µL of **Wash Solution A** to each well of the 96-Well Isolation Plate (Deep Well). Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire **Wash Solution A** has passed through into the collection/waste tray by inspecting the 96-Well Isolation Plate (Deep Well). If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

e. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
f. Repeat steps **3d** and **3e** to wash column for a second time using Wash Solution A.
g. Pat the bottom of the 96-Well Isolation Plate (Deep Well) dry. Reassemble the 96-Well Isolation Plate (Deep Well) and the vacuum manifold. Apply vacuum for an additional 15 minutes in order to completely dry the plate.
h. Turn off vacuum and ventilate the manifold.

4. RNA Elution

a. Replace the collection/waste tray in the vacuum manifold with the provided 96-Well Elution Plate (Deep Well). Complete the vacuum manifold assembly with the 96-Well Isolation Plate (Deep Well).
b. Add 75 µL of **Elution Solution A** to each well of the plate.
c. Apply vacuum for 5 minutes.

5. Storage of RNA

Use the provided adhesive tape to seal the 96-Well Elution Plate (Deep Well). The purified RNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

B. Total RNA Purification from All Types of Lysate Using Centrifugation

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

2. Binding RNA to 96-Well Isolation Plate (Deep Well)

a. Place the 96-Well Isolation Plate (Deep Well) on top of a provided 96-Well Collection Plate (Deep Well).
b. Apply up to 600 µL of the lysate with the ethanol (from **Step 1**) into each well of the 96-Well Isolation Plate (Deep Well). Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application to the wells

c. Discard the flowthrough. Reassemble the 96-Well Isolation Plate (Deep Well) and the bottom plate.
d. Repeat Step **2b** and **2c** as necessary to bind the remaining lysate volume.

Note: Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

3. RNA Wash

a. Apply 400 µL of **Solution WN** to each well of the 96-Well Isolation Plate (Deep Well). Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

Note: Ensure the entire Wash Solution A has passed through into the bottom plate by inspecting the 96-Well Isolation Plate (Deep Well). If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Isolation Plate (Deep Well) and the bottom plate.

c. Apply 400 µL of **Wash Solution A** to each well of the 96-Well Isolation Plate (Deep Well). Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes. Discard the flowthrough. Reassemble the 96-Well Isolation Plate (Deep Well) and the bottom plate.

Note: Ensure the entire **Wash Solution A** has passed through into the bottom plate by inspecting the 96-Well Isolation Plate (Deep Well). If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

d. Repeat steps **3c** to wash column for a second time with Wash Solution A.

e. Pat the bottom of the 96-Well Isolation Plate (Deep Well) dry. Reassemble the 96-Well Isolation Plate (Deep Well) and the bottom plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 15 minutes in order to completely dry the plate.

4. RNA Elution

a. Stack the 96-Well Isolation Plate on top of the 96-Well Elution Plate (Deep Well).

b. Add 75 µL of **Elution Solution A** to each well of the 96-Well Isolation Plate (Deep Well).

c. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 5 minutes.

5. Storage of RNA

Use the provided adhesive tape to seal the 96-Well Elution Plate (Deep Well). 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.

Technical Support

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.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at support@norgenbiotek.com.

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

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