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# Saliva / Swab RNA Purification Kit Dx

# Product Insert

REF Dx69100 CE IVD PIDx69100-4

## Intended Use

Norgen's Saliva/Swab RNA Purification Kit Dx 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 Dx (Cat.53800) or preserved swabs collected in Norgen's Total Nucleic Acid Preservative Tubes Dx (Cat. Dx69200). The purified RNA is intended for *in vitro* diagnostic use for medical purposes.

**For *In Vitro* Diagnostic Use**

## Product Description

Norgen's Saliva/Swab RNA Purification Kit Dx 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 Dx (Cat. 53800) or preserved swabs collected in Norgen's Total Nucleic Acid Preservative Tubes Dx (Cat. Dx69200). 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 chemistry employed in the kit allows the purification of total RNA, including viral and bacterial RNA, irrespective of size or GC content. The purified RNA is ideal for *in vitro* diagnostic use for medical purposes.

This kit is optimized to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated using the RNA isolated with Norgen's Saliva / Swab RNA Purification Kit Dx in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's Saliva / Swab RNA Purification Kit Dx is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques.

Norgen's Saliva / Swab RNA Purification Kit Dx does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

## Kit Components

| Component | Product # Dx69100 (50 preps) |
|---|---|
| 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 |

**Label Legend**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  |  |
| Do not reuse | Use by | Batch Code | Catalogue Number | Contains sufficient for <n> tests | Manu-facturer | In Vitro Diagnostic Medical Device | Consult instructions for use | Temper-ature limitation |

**Advantages**

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Isolate high quality total RNA, including viral RNA, from fresh and preserved saliva and swab samples
- Fits into *in vitro* diagnostic workflows
- Fast and easy processing using rapid spin-column format
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of sources
- RNA can be isolated and detected from as little as a single animal cell

**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 *<br>*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.

**Warnings and Precautions**

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 Dx:

*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 Dx (53800)

*For Preserved Nasal or Throat Swabs*

- Norgen's Total Nucleic Acid Preservative Tubes Dx (Cat. Dx69200)
- Sterile nylon flocked swabs

*For Non-Preserved Nasal or Throat Swabs*

- Sterile nylon flocked swabs

## 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.

**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**

- 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 Dx (Cat. 53800) 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 Dx (Cat. Dx69200) 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 Dx (Cat. Dx69200).
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 **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 **2b** and **2c** as necessary to bind the remaining lysate volume.

**Optional Step:**
Norgen's Saliva/Swab RNA Purification Kit Dx 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 **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.

**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 Dx isolates 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).

**Product Use Restriction**

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The respective user is liable for any and all damages resulting from application of Norgen's Saliva/Swab RNA Purification Kit Dx for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

**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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