

## Saliva DNA Isolation Kit Dx

**REF** Dx45400



**IVD**

## Product Insert

 PIDx45400-6

### Intended Use

Norgen's Saliva DNA Isolation Kit Dx provides a fast and simple procedure for isolating genomic DNA from saliva samples collected and preserved using Norgen's Saliva DNA Collection and Preservation Devices, as well as fresh saliva for subsequent *in vitro* diagnostic use. Purification is based on spin column chromatography. The genomic DNA is preferentially purified from other cellular components such as proteins and RNA. Saliva DNA can either be isolated from saliva samples collected and preserved using Norgen's Saliva Collection and Preservation Devices or fresh saliva samples.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated using the DNA isolated with Norgen's Blood Saliva DNA Isolation 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 DNA Isolation Kit Dx is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques including DNA isolation.

Norgen's Saliva DNA Isolation 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 #Dx45400 (50 samples)
Lysis Solution	30 mL
Proteinase K in Storage Buffer	1.2 mL
Binding Solution	12 mL
Wash Solution	18 mL
Elution Buffer	12 mL
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

### Label Legend

		<b>LOT</b>	<b>REF</b>			<b>IVD</b>		
Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manufacturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temperature limitation

## Advantages

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Fits into *in vitro* diagnostic workflows
- Sample collection is non-invasive and painless
- Fast and easy processing using a rapid spin-column format
- Isolate high quality genomic DNA
- Compatible with preserved saliva samples collected using Norgen's Saliva DNA Collection and Preservation Devices (please see Related Products Table)

## Specifications

Kit Specifications	
Number of preps	50
Maximum Saliva Input	0.5 mL preserved saliva 0.25 mL fresh saliva
Average Yield from 0.25 mL of Saliva*	3 -7 µg
Average purity (OD260/280)	1.7 - 2.1
Time to Complete 10 Purifications	30 minutes

\* Average DNA yield will vary depending on the donor

## Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. All solutions and plastics can be used until the expiration date specified on their labels.

## 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 Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

The **Binding Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Saliva 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.

## Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- Water (for rinsing mouth)
- Norgen's Saliva DNA Collection and Preservation Devices (optional)
- 96 - 100% ethanol
- Isopropanol
- 55°C Incubator

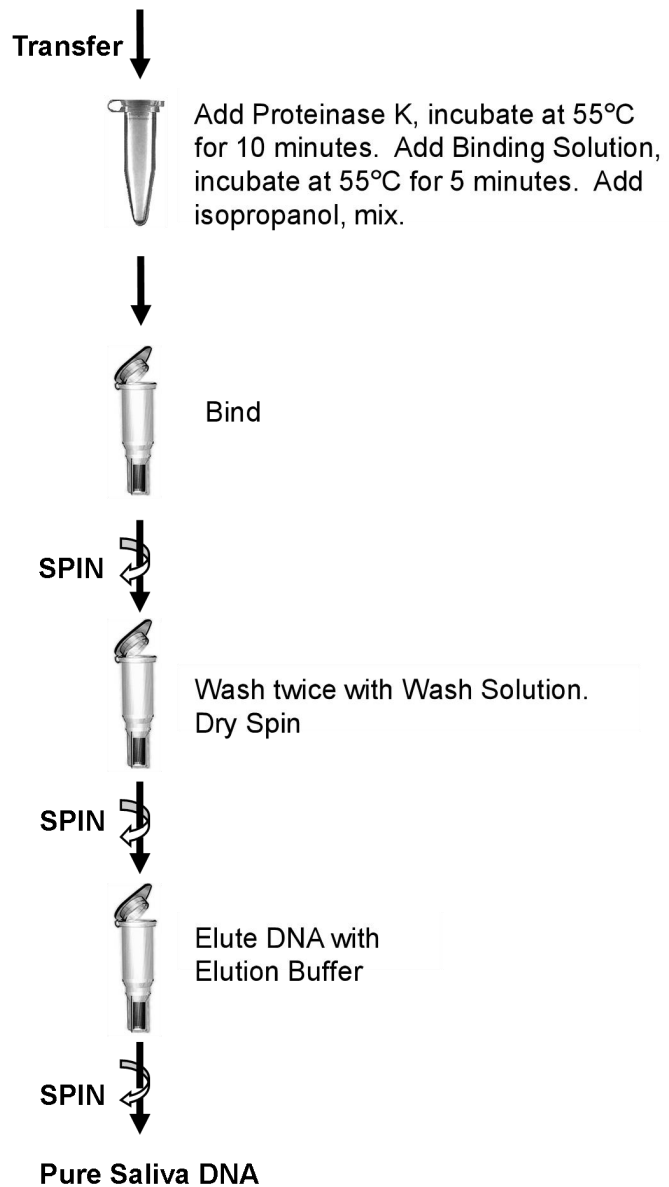
## Procedure

All centrifugation steps are carried out at room temperature in a benchtop microcentrifuge that has a maximum speed 20,000 x g (approximately 14,000 rpm). Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds.

### Flow Chart

Procedure for Purifying Saliva DNA using Norgen's Saliva DNA Isolation Kit Dx

Preserved Saliva Samples collected using Norgen's Saliva DNA Collection and Preservation Devices, or a mixture of fresh saliva and Lysis Solution. Mix.



## Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Saliva samples should be collected and preserved using Norgen's Saliva DNA Collection and Preservation Devices (please see Related Products Table).
- Prepare a working concentration of **Wash Solution** by adding 42 mL of 96 -100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- The protocol provided is for isolation of DNA from 500  $\mu$ L of preserved saliva. If a starting volume of less than 250  $\mu$ L is preferred, scale down the amount of **Proteinase K**, **Binding Buffer B** and **Isopropanol** used in Section 1 proportionally.
- All centrifugation steps are performed at room temperature.
- **Always** vortex the Proteinase K before use.

## 1. Saliva Sample Collection and Lysate Preparation

### A. Samples collected using Norgen's Saliva DNA Collection and Preservation Devices

- a. Collect and preserve saliva samples using Norgen's Saliva DNA Collection and Preservation Devices (please see Related Products Table).
- b. Before using the preserved saliva for DNA isolation, mix the preserved saliva by inversion for a few seconds.
- c. Aliquot 500  $\mu$ L of preserved saliva to a microcentrifuge tube.
- d. Add 20  $\mu$ L of **Proteinase K** (vortex before use). Mix by vortexing and incubate at 55°C for 10 minutes.
- f. Add 200  $\mu$ L of **Binding Solution** to the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.
- g. Add 720  $\mu$ L of Isopropanol and mix by vortexing for a few seconds.
- h. Proceed to Section 2: Sample Binding to Column.

### B. Fresh Saliva Samples

- a. Prior to collection of saliva samples, the donor should rinse their mouth with a few millilitres of water for 10 seconds in order to remove any food particles that may be present. If food particles are present they may cause clogging of the column.
- b. Ten minutes after rinsing collect saliva by spitting into a sterile collection tube or vial (not provided). The amount of saliva collected should be at least 100  $\mu$ L but not more than 2 mL.
- c. Transfer 250  $\mu$ L of liquid saliva to a sterile microcentrifuge tube.
- d. Add 250  $\mu$ L **Lysis Solution**, and mix by vortexing.
- e. Add 20  $\mu$ L of **Proteinase K** (vortex before use). Mix by vortexing and incubate at 55°C for 10 minutes.
- f. Add 200  $\mu$ L of **Binding Solution** the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.
- g. Add 720  $\mu$ L of Isopropanol and mix by vortexing for a few seconds.
- h. Proceed to Section 2: Sample Binding to Column.

## 2. Sample Binding to Column

- a. Assemble a column with one of the provided collection tubes.
- b. Apply up to 760  $\mu$ L of the lysate to the column and centrifuge for 1 minute at 6,000 RPM (~3800 x g).

**Note:** Ensure the entire sample has passed through into the collection tube by inspecting the column. If the entire sample volume has not passed, spin for additional 1 minute.

- c. Discard the flowthrough and reassemble the spin column with its collection tube.

**Note:** Each spin column provided is capable of processing up to 500  $\mu$ L of lysed saliva. If additional DNA isolation is desired, use an additional spin column. For example, if 1 mL of saliva is to be processed, use two spin columns and process 500  $\mu$ L of lysed saliva with each column.

- d. Repeat **Steps 2b and 2c** until all the lysate has passed through the column.

### 3. Column Wash

- a. Apply 500  $\mu$ L of **Wash Solution** to the column and centrifuge for 1 minute at 8,000 RPM (~6800 x g). Discard the flowthrough and reassemble the spin column with its collection tube.

**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. Apply 500  $\mu$ L of **Wash Solution** to the column and centrifuge for 1 minute at maximum speed. Discard the flowthrough and reassemble the spin column with its collection tube.  
c. Spin the column for 2 minutes at maximum speed in order to thoroughly dry the column. Discard the collection tube.

### 4. DNA Elution

- d. Place the column into a fresh 1.7 mL Elution tube provided with the kit.  
e. Add 100  $\mu$ L of **Elution Buffer** to the column. Incubate for 5 minutes at 55°C.  
f. Centrifuge for 2 minutes at 2,000 RPM (~ 425x g), followed by a second spin for 1 minute at Maximum speed.

**Note:** For more concentrated sample, 50  $\mu$ L Elution Buffer can be used.

### 5. Storage of DNA

The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at -20°C for long-term storage.

Related Products	Product #
Saliva DNA Collection and Preservation Devices (50)	49000
Saliva DNA Collection, Preservation and Isolation Kit	35700

## Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The micro spin column is clogged.	Centrifugation speed was too low or spin time was inadequate.	Check the centrifuge to ensure that it is capable of generating the required RPMs. Sufficient centrifugal force is required to move the liquid phase through the column. Also ensure that the correct spin times are followed. Spinning for a few additional minutes will help.
	The sample is too large	Too many cells were applied to the column. Ensure that no more than 0.5 mL of preserved saliva is applied to the column. Clogging can be alleviated by centrifuging for a longer period of time until the lysate passes through the column.
	The lysate/binding solution mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the spin column.
The yield of genomic DNA is low	Incomplete lysis of cells	Increased Proteinase K incubation time at 55°C may result in increased yields
	The DNA elution is incomplete	Perform an additional centrifugation of 2 minutes at 14,000 x g to ensure that all the DNA is eluted.
	DNA concentration in the saliva sample being used is low.	Some saliva samples contain very little DNA. This varies from individual to individual based on numerous variables. Increased proteinase K incubation time at 55°C may result in increased yields.
DNA does not perform well in downstream applications.	DNA was not washed with the provided Wash Solutions	Traces of salt from the binding step may remain in the sample if the column is not washed with the Wash Solutions. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is present in eluted DNA.	RNA is coeluted with the DNA.	Carry out a digestion with RNase A on the elution if the RNase present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature.

### Product Use Restriction

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

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Norgen's Saliva DNA Isolation 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.

The respective user is liable for any and all damages resulting from application of Norgen's Saliva DNA Isolation 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](http://www.norgenbiotek.com)) or through email at [support@norgenbiotek.com](mailto:support@norgenbiotek.com).

### Authorized Representative



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