

## Saliva DNA Isolation Kit – 50 Preps

Product #45400

## Product Insert

Norgen's Saliva DNA Isolation Kit 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. Human genomic DNA extracted from buccal epithelial cells and white blood cells found in saliva can be used in various applications in diagnostics. The isolated DNA can be used for the detection of biomarkers to diagnose a disease, follow the diseases progress or monitor the effects of a particular treatment. Saliva DNA can also be used to diagnose particular types of infections. Isolation of DNA from saliva has become an attractive alternative to isolation from blood or tissue due to the fact that sample collection is non-invasive, the samples can be collected by individuals with little training, and no special equipment is required. Saliva DNA purified using Norgen's kit is of the highest quality, and is compatible with a number of downstream applications including PCR, Southern Blot analysis, sequencing and microarray analysis. This kit can be used to isolate saliva DNA for *in vitro* diagnostic use.

### Norgen's Purification Technology

Purification is based on spin column chromatography. The genomic DNA is preferentially purified from other cellular components such as proteins and RNA. The process first involves the collection and preservation of the saliva samples using Norgen's Saliva DNA Collection and Preservation Devices. The resulting saliva sample can be processed immediately, or alternatively can be stored at room temperature for over 2 years prior to DNA isolation. When DNA isolation is required, Proteinase K and Binding Solution are then added to the sample, followed by the addition of ethanol. The resulting solution is loaded onto a spin-column. Only the DNA will bind to the column, while most of the RNA and proteins will be removed in the flowthrough. The bound DNA is then washed twice with the provided Wash Buffer in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer. The purified DNA is of the highest quality and can be used in a number of downstream applications.

### Specifications

Kit Specifications	
Number of preps	50
Maximum Saliva Input	0.5 mL of preserved saliva
Average Yield from 0.25 mL of Saliva*	7 µg
Average purity (OD260/280)	1.8 - 2.1
Time to Complete 10 Purifications	90 minutes (30 minutes hands on)

\* Average DNA yield will vary depending on the donor

### Storage Conditions and Product Stability

- The kit contains ready-to-use **Proteinase K** which is dissolved in a specially prepared storage buffer. The Proteinase K should be stored at room temperature or 4°C up to the expiry date indicated on the label.
- All other solutions should be kept tightly sealed and stored at room temperature (15 – 25 °C) up to the expiry date indicated on the label without any reduction in kit performance.

### Advantages

- 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)

### Kit Components

Component	Product #45400 (50 samples)
Proteinase K	0.6 mL
Binding Solution	36 mL
Wash Solution	18 mL
Elution Buffer	12 mL
Micro Spin Columns	50
Elution tubes (1.7 mL)	50
Product Insert	1

### Quality Control

In accordance with Norgen's Quality Management System, each lot of Norgen's Saliva DNA Isolation Kits is tested against predetermined specifications to ensure consistent product quality.

### Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use. We reserve the right to change, alter, or modify any product to enhance its performance and design.

### Precautions and Disclaimers

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.

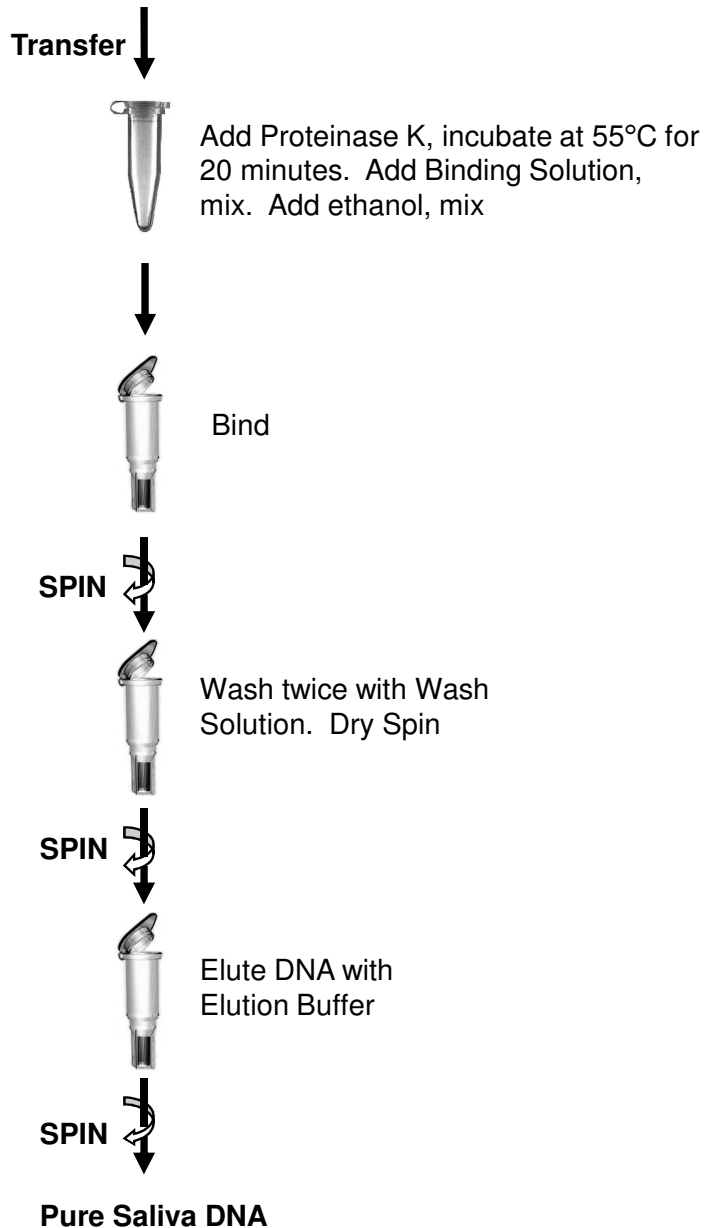
### Customer-Supplied Reagents and Equipment

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

## Flow Chart

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

Preserved Saliva Samples collected using  
Norgen's Saliva DNA Collection and Preservation Devices.  
Mix, incubate at 55°C for 1 hour. Mix.



## 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.

### 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 µL of preserved saliva. If a starting volume of less than 500 µL is preferred, scale down the amount of **Proteinase K**, **Binding Solution**, and 96 – 100% Ethanol used in Section 1 proportionally.
- All centrifugation steps are performed at room temperature.

### 1. Saliva Sample Collection and Lysate Preparation

- a. Collect and preserve saliva samples using Norgen's Saliva DNA Collection and Preservation Devices (please see Related ProductsTable).
- b. Before using the preserved saliva for DNA isolation, mix the preserved saliva by inversion for a few seconds.
- c. Incubate the preserved saliva at 55°C for a minimum of 1 hour. Mix the preserved saliva by inversion for a few seconds after the incubation.
- d. Aliquot 500 µL of preserved saliva to a microcentrifuge tube.
- e. Add 10 µL of **Proteinase K**. Mix by vortexing and incubate at 55°C for 20 minutes.
- f. Add 250 µL of **Binding Solution** the saliva sample. Mix by vortexing.
- g. Add 750 µL of 96 -100 % Ethanol and mix by vortexing for a few seconds.

### 2. Sample Binding to Column

- a. Apply up to 760 µL of the lysate from **Step 1g** to the column (assembled with its collection tube) and centrifuge for 1 minute at 8,000 x g (~8,000 RPM).

**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.

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

**Note:** Each spin column provided is capable of processing up to 500  $\mu\text{L}$  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  $\mu\text{L}$  of lysed saliva with each column.

- c. Repeat **Steps 2a and 2b** to complete the binding of the lysate to the column.

### 3. Column Wash

- a. Apply 500  $\mu\text{L}$  of **Wash Solution** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). 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. Wash column a second time by adding another 500  $\mu\text{L}$  of **Wash Solution** and centrifuging for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Spin the column for 2 minutes at 14,000 x g (~14,000 RPM) in order to thoroughly dry the column. Discard the collection tube.

### 4. DNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 200  $\mu\text{L}$  of **Elution Buffer** to the column. Incubate for 1 minute.
- c. Centrifuge for 1 minutes at **14,000 x g (~14,000 RPM)**.

**Note:** For more concentrated sample, 100  $\mu\text{L}$  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

### 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](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

## 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 two times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed two times with the Wash Solution. 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.

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