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Saliva DNA Isolation Kit – 50 Preps — Product Insert

Product #RU45400

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, as well as fresh saliva. 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 research applications including PCR, Southern Blot analysis, sequencing and microarray analysis.

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. Saliva DNA can either be isolated from saliva samples collected and preserved using Norgen's Saliva Collection and Preservation Devices or fresh saliva samples. Preserved saliva samples (fresh saliva samples are mixed with Lysis Buffer F) are mixed with Proteinase K and incubated for 10 minutes at 55°C, then Binding Buffer B is added to the sample, followed by a second incubation for 5 minutes at 55°C. Isopropanol is then added to the mixture. The resulting solution is then 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 flowthough. The bound DNA is then washed with the provided Wash Solutions in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer B. 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 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

The kit contains ready-to-use **Proteinase K** which is dissolved in a specially prepared storage buffer. All solutions should be kept tightly sealed and stored at room temperature (15 – 25°C). This kit is stable for 2 years after the date of shipment.

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 #RU45400 (50 samples)
Lysis Buffer F	30 mL
Proteinase K in Storage Buffer	1.2 mL
Binding Buffer B	12 mL
Wash Solution A	18 mL
Elution Buffer B	15 mL
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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

Binding Buffer B 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

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, or a mixture of fresh saliva and Lysis Buffer F.

Transfer

Add Proteinase K, incubate at 55°C for 10 minutes. Add Binding Buffer B, incubate at 55°C for 5 minutes. Add isopropanol, mix

Bind

SPIN

Wash twice with Wash Solution A. Dry Spin

SPIN

Elute DNA with Elution Buffer B

SPIN

Pure Saliva DNA

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.

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 A** by adding 42 mL of 96- 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution A**. 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 250 µL 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

IMPORTANT NOTE: For Norgen Saliva DNA Collection and Preservation Devices purchased before January 2020, please follow the procedure below. For Norgen Saliva DNA Collection and Preservation Devices purchased after January 2020, vortex the Collection Tube containing preserved saliva for 10 seconds and incubate at 55°C for one hour, then proceed directly to Step f below.

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 µL of preserved saliva to a microcentrifuge tube.
d. Add 20 µL of **Proteinase K** (vortex before use). Mix by vortexing and incubate at 55°C for 10 minutes.
f. Add 200 µL of **Binding Buffer B** to the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.
g. Add 720 µL of Isopropanol and mix by vortexing for a few seconds.
h. Proceed to Section 2: Saliva DNA isolation.

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 µL but not more than 2 mL.
c. Transfer 250 µL of liquid saliva to a sterile microcentrifuge tube.
d. Add 250 µL **Lysis Buffer F**, and mix by vortexing.
e. Add 20 µL of **Proteinase K** (vortex before use). Mix by vortexing and incubate at 55°C for 10 minutes.
f. Add 200 µL of **Binding Buffer B** the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.

g. Add 720 μL of Isopropanol and mix by vortexing for a few seconds.
h. Proceed to Section 2: Saliva DNA isolation

2. Saliva DNA Isolation

a. Assemble a column with one of the provided collection tubes.
b. Apply up to 760 μL 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 μL 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 μL of lysed saliva with each column.

d. Repeat Steps **2b** and **2c** until all the lysate has passed through the column.
e. Apply 500 μL of **Wash Solution A** 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.

f. Apply 500 μL of **Wash Solution A** to the column and centrifuge for 1 minute at maximum speed. Discard the flowthrough and reassemble the spin column with its collection tube.
g. Spin the column for 2 minutes at maximum speed in order to thoroughly dry the column. Discard the collection tube.
h. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
i. Add 100 μL of **Elution Buffer B** to the column. Incubate for 5 minutes at 55°C.
j. Centrifuge for 2 minutes at 2,000 RPM (~ 425 x g), followed by a second spin for 1 minute at maximum speed.

Note: For a more concentrated sample, 50 μL Elution Buffer B can be used.

k. 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.

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 55ºC 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 55ºC may result in increased yields.
DNA does not perform well in downstream applications.	DNA was not washed with Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin after the column wash steps 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.

Related Products	**Product #**
Saliva DNA Collection and Preservation Devices (50)	RU49000
Saliva DNA Collection, Preservation and Isolation Kit	RU35700

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 techsupport@norgenbiotek.com.

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