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Saliva Collection and DNA Isolation Kit — Product Insert

Product #21400

Norgen's Saliva Collection and DNA Isolation Kit provides a fast and simple procedure for isolating genomic DNA from up to 500 µL of saliva. The Saliva Collection Tubes provided with the kit contain preservatives that allow for the storage of the saliva samples for extended periods of time at room temperature prior to DNA isolation. Human genomic DNA extracted from buccal epithelial cells and white blood cells found in saliva can be used in various applications in diagnostics. The 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. The 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.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The genomic DNA is preferentially purified from other cellular components such as proteins and RNA. The process involves first mixing the saliva sample, collected either by spitting or cotton swab, with the preservatives contained in a provided **Saliva Collection Tube** (please see the flow chart on page 3). The resulting preserved saliva is stable for an extended period of time even at room temperature, and is resistant to further bacterial contamination. When DNA isolation is required, binding solution is then added to the samples and the solution is loaded onto a spin-column. Norgen's resin binds DNA in a manner that depends on ionic concentrations. Thus 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 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	
Maximum Saliva Input	0.5 mL
Average Yield from 0.5 mL of Saliva	5 µg
Time to Complete 10 Purifications	30 minutes

Advantages

- Saliva samples are stable for long periods at room temperature due to preservatives in the Saliva Collection Tube
- Sample collection is non-invasive and painless
- Fast and easy processing using a rapid spin-column format
- DNA can be isolated and detected from as little as 100 µL of saliva
- Isolate high quality genomic DNA

Kit Components

Component	Product #21400 (25 samples)
Saliva Collection Tubes	25
Binding Solution	6 mL
Wash Solution	9 mL
Elution Buffer	8 mL
Micro Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

Storage Conditions and Product Stability

All **Saliva Collection Tubes** and solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

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*.

The **Binding Solution** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride 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)
- Nuclease-free molecular biology-grade water (for cotton swab method)
- 96 - 100% ethanol

Flow Chart

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

Collect saliva sample into **Saliva Collection Tube**

Transfer

Add Binding Solution,
Vortex to mix

Bind

SPIN

Wash twice with
Wash Solution

SPIN

Elute DNA with
Elution Buffer

SPIN

Pure Saliva DNA

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.
- After saliva samples have been collected in the **Saliva Collection Tubes** they may be stored at room temperature for up to 1 month, or frozen at -20°C for longer-term storage.
- Prepare a working concentration of **Wash Solution** by adding 21 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 30 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.

1A. Saliva Sample Collection and Preservation - Spitting

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 provided **Saliva Collection Tube**. The amount of saliva collected should be at least 100 µL but not more than 2 mL (as indicated by the marks on the collection tube).

c. Close the lid of the **Saliva Collection Tube**. Mix the saliva and the preservatives by vortexing. Ensure that the preservatives are dissolved completely for optimal preservation. Proceed to **Step 2**.

Note: At this point, the saliva samples may be stored at room temperature for storage up to 1 month, or frozen at -20°C for longer-term storage.

1B. Saliva Sample Collection and Preservation – Cotton Swab

a. Aliquot 500 µL of nuclease-free molecular biology-grade water into a provided **Saliva Collection Tube**. Mix the saliva and the preservatives by vortexing. Ensure that the preservatives are dissolved completely for optimal preservation.

b. Gently brush a sterile, single-use cotton swab inside the mouth along the cheek

c. Using sterile techniques, cut the cotton tip where the buccal cells were collected and place into the **Saliva Collection Tube** with dissolved preservatives. Close the lid of the tube. Vortex gently and incubate for 5 minutes at room temperature. Proceed to **Step 2**.

Note: At this point, the saliva samples may be stored at room temperature for storage up to 1 month, or frozen at -20°C for longer-term storage.

2. Sample Binding to Column

a. Vortex the preserved saliva from Step **1A** or Step **1B** for 10 seconds. Transfer up to 500 µL of preserved saliva to a sterile microcentrifuge tube (not provided). Add 160 µL of **Binding Solution**. Mix by vortexing.

Note: Each spin column provided is capable of processing up to 500 µL of preserved 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 preserved saliva with each column.

b. Assemble a spin column with a provided collection tube. Apply the saliva mixture to the column and centrifuge for 2 minutes at 14,000 x g (~14,000 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 2 minutes.

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

3. Column Wash

a. Apply 500 µL of **Wash Solution** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).

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 µL of **Wash Solution** and centrifuging for 2 minutes at 14,000 x g (~14,000 RPM).
c. Ensure that the column is dry. Spin for an additional minute, if necessary.
d. Discard the collection tube with the flowthrough.

4. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 100 µL of **Elution Buffer** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~14,000 RPM).** Note the volume eluted from the column. If the entire 100 µL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 4b** and **4c**).

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.

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 resin. 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 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	An incubation at 60ºC for 15 minutes after the addition of Binding Solution may result in increased yields
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 x g is performed after the 200 x g centrifugation cycle, 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. An incubation at 60ºC for 15 minutes after the addition of Binding Solution may result in increased yields.
DNA is degraded	Preservative was not completely mixed with the saliva sample.	Ensure that the preservative was mixed well with the saliva sample. Heating at 60^{o}C for 5 minutes may aide the process.

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications.	DNA was not washed twice with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed twice with 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.

Related Products	Product #
ProteoSpin™ Urine Protein Concentration Micro Kit	17400
Urine DNA Isolation Kit	18100
Saliva DNA Isolation Kit	21410

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) or through email at techsupport@norgenbiotek.com.

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