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Saliva DNA Isolation 96-Well Kit — Product Insert

Product # RU35200

Norgen's Saliva DNA Isolation 96-Well Kit provides a rapid method for the high-throughput isolation of total genomic DNA from saliva. Human genomic DNA extracted from buccal epithelial cells and white blood cells found in saliva is of the highest integrity and can be used in various downstream applications such as real time PCR, Southern blotting, sequencing, macro/micro array and SNP assays. Purification can be performed on either a vacuum manifold or using centrifugation. This product is compatible with Norgen's Saliva DNA Collection and Preservation Kit, as well as Norgen's Buccal DNA Collection and Preservation Kit.

Advantages

- Sample collection is non-invasive and painless
- Fast and easy high throughput processing using 96-well plates
- Process using a centrifuge or vacuum manifold
- DNA can be isolated and detected from as little as 100 µL of saliva
- Isolate high quality genomic DNA

Specifications

Kit Specifications	
Binding Capacity Per Well	50 µg
Maximum Saliva Input	0.5 mL of preserved saliva
Average Yield from 0.25 mL of Saliva*	3 - 5 µg
Average Purity (OD 260/280)	1.7
Time to Complete 96 Purifications	40 minutes

Kit Components

Component	Product # RU35200 (192 preps)
Lysis Buffer F	100 mL
Proteinase K in Storage Buffer	4 mL
Binding Buffer B	40 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	30 mL
96-Well Plate	2
96-Well Collection Plate	2
Adhesive Tape	4
96-Well Elution Plate	2
Product Insert	1

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. All other solutions should be kept tightly sealed and stored at room temperature (15 – 25°C). This kit is stable for 1 year after the date of shipment.

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 form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

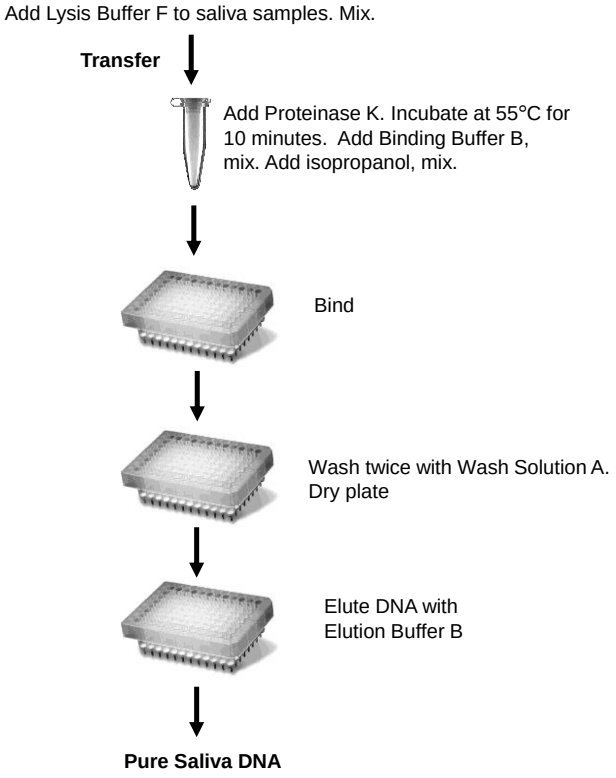
Saliva of all human and animal subjects is considered potentially infectious. However, Norgen's preserved saliva is non-infectious, for all microorganisms are lysed. Nonetheless, All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with saliva.

Customer-Supplied Reagents and Equipment

- Micropipettors and multichannel pipettes
- 96-100% ethanol
- Isopropanol
- 55°C Incubator
- Collection/Waste Tray for vacuum manifold.
- For ***Vacuum Format***:
    - Vacuum manifold with vacuum pump capable of generating a minimum vacuum of -650 mbar or -25 in. Hg.
    - Sealing tape or pads
- For ***Centrifuge Format***:
    - Centrifuge with rotor for 96-well plate assembly, such as AllSpin Js-5.3 Rotor for Avanti® J-26xp centrifuge, Beckman Coulter or similar rotor that can hold the stack of the 96-well plate and the 96-Well Collection Plate and that can reach the minimum speed of 4000 rpm (~4000xg)

Flowchart

Procedure for Purifying Total DNA using Norgen's Saliva DNA Isolation 96-Well Kit



Procedures

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: All centrifugation steps are carried out at room temperature in a conventional laboratory centrifuge using a swing bucket rotor capable of 3000-5000 x g (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R).

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Freshly collected, preserved or frozen saliva (without preservation) may be used as a starting material.
- Prepare a working concentration of **Wash Solution A** by adding 90 mL of 96-100% ethanol (to be provided by the user) to the supplied bottles containing concentrated **Wash Solution A**. This will give a final volume of 128 mL. The labels on the bottles have a box that can be checked to indicate that ethanol has been added.
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- The maximum recommended input of lysed saliva mixture is 500 µL per well of the 96-Well Plate.

1A. Saliva Sample Collection and Lysis - 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 wells.
b. Ten minutes after rinsing collect saliva by spitting into a sterile collection tube or vial (not provided).
c. Transfer 250 µL of saliva to a sterile tube and add 250 µL of **Lysis Buffer F.** Vortex the saliva sample for 10 seconds.
d. Add 20 µL of Proteinase K. Mix by vortexing and incubate at 55°C for 10 minutes.
e. Add 200 µL of **Binding Buffer B** to the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.
f. Add 720 µL of Isopropanol and mix by vortexing for a few seconds.
g. Proceed to Step 2, Total DNA Isolation.

1B. Norgen's Saliva DNA Collection and Preservation Device

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, vortex the saliva sample for 10 seconds.
c. Transfer 500 µL of preserved saliva to a clean tube.
d. Add 20 µL of Proteinase K. Mix by vortexing and incubate at 55°C for 10 minutes.
e. Add 200 µL of **Binding Buffer B** to the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.
f. Add 720 µL of Isopropanol and mix by vortexing for a few seconds.
g. Proceed to Step 2, Total DNA Isolation.

1C. Saliva Sample Collection and Lysis – Cotton Swab

a. Aliquot 500 µL of **Lysis Buffer F** into a sterile microcentrifuge tube (not provided).
b. Gently place one swab in cheek pouch, rotate and move the swab head for 30 seconds to collect as much saliva as possible.

c. Using sterile techniques, clip the swab head into the microcentrifuge tube with Lysis Buffer F. If possible, repeat steps using a second swab.
d. Close the lid of the tube. Vortex gently and incubate for 5 minutes at room temperature.
e. Add 20 µL of Proteinase K. 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 Step 2, Total DNA Isolation.

1D. Buccal Cells Sample Collection and Lysis – Cotton Swab

a. Aliquot 500 µL of **Lysis Buffer F** into a sterile microcentrifuge tube (not provided).
b. Gently brush a sterile, single-use cotton swab inside the mouth along the cheek.
c. Clip the cotton tip where the buccal cells were collected and place into the microcentrifuge tube with **Lysis Buffer F**. If possible, repeat steps using a second swab.
d. Close the lid of the tube. Vortex gently and incubate for 5 minutes at room temperature.
e. Add 20 µL of Proteinase K. 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 Step 2, Total DNA Isolation.

2. Total DNA isolation

Note: The isolation of DNA from saliva can be performed using either a vacuum manifold or centrifugation. For purification using vacuum follow the procedure in Section 2A; and for purification using centrifugation, follow the procedure outlined in Section 2B.

A. Total DNA Isolation Using Vacuum Manifold

1. Binding DNA to 96-Well Plate

a. Apply up to 750 µL of the lysate into each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
b. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Plate and the vacuum manifold.

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

c. Perform a second binding step as outlined in **Step 1a**.
d. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Plate and the vacuum manifold.

2. DNA Wash

a. Apply 500 µL of **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire volume of **Wash Solution A** has passed through into the collection/waste tray by inspecting the 96-Well Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Plate and the vacuum manifold.
d. Repeat **Step 2a** to wash the plate a second time.
e. Turn off vacuum and ventilate the manifold. Discard the flowthrough

f. Pat the bottom of the 96-Well Plate gently to remove any residual wash buffer. Reassemble the 96-Well Plate and the vacuum manifold. Apply vacuum for an additional 10 minutes in order to completely dry the plate.
g. Turn off vacuum and ventilate the manifold.
h. Pat the bottom of the 96-Well Plate gently to remove any residual wash buffer.

3. DNA Elution

a. Replace the collection/waste tray in the vacuum manifold with the provided 96-Well Elution Plate. Complete the vacuum manifold assembly with the 96-Well Plate.
b. Add 100 µL of **Elution Buffer B** to each well of the plate.
c. Apply vacuum for 5 minutes.
d. Store purified gDNA at –20°C for a few days or at –70°C for long term storage.

B. Total DNA Isolation Using Centrifugation

1. Binding DNA to 96-Well Plate

a. Place the 96-Well Plate on top of a provided 96-Well Collection Plate.

Note: The user should ensure that the assembled 96-Well Plate and the 96-Well Collection Plate stack fits into the rotor without interfering with the centrifugation process.

b. Apply up to 750 µL of the mixture into each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 x g (~4,000 RPM) for 2 minutes.
c. Discard the flowthrough. Reassemble the 96-Well Plate and the 96-Well Collection Plate.

Note: Ensure that all of the lysate from each well has passed through into the 96-Well Collection Plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

d. Perform a second binding step as outlined in **Step 1b**.
e. Discard the flowthrough. Reassemble the 96-Well Plate and the 96-Well Collection Plate.

2. DNA Wash

a. Apply 500 µL of **Wash Solution A** to each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 x RPM (~3420 x g) for 2 minutes.

Note: Ensure the entire volume of Solution WN has passed through into the 96-Well Collection Plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Plate and the 96-Well Collection Plate.
c. Repeat **Step 2a** to wash the plate a second time.
d. Discard the flowthrough. Pat the bottom of the 96-Well Plate dry. Reassemble the 96-Well Plate and the 96-Well Collection Plate. Centrifuge the assembly at a minimum speed of 4,000 x RPM (~3420 x g) for 15 minutes in order to completely dry the plate.

3. DNA Elution

a. Stack the 96-Well Plate on top of the 96-Well Elution Plate.
b. Add 100 µL of **Elution Buffer B** to each well of the 96-Well Plate.
c. Centrifuge the assembly at a minimum speed of 2,000 x g (~800 RPM) for 2 minutes followed by 4,000 x RPM (~3420 x g) for 3 minutes.
d. Store purified gDNA at –20°C for a few days or at –70°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Clogged Wells in Plate	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog.
	The sample is too large	Ensure that no more than 0.5 mL of lysed saliva is applied during each binding step. Clogging can be alleviated by vacuuming or centrifuging for a longer period of time until the lysate passes through the wells of plate.
The yield of genomic DNA is low	Incomplete lysis of cells	Increased Proteinase K incubation time at 55ºC may result in increased yields
	DNA concentration in the saliva sample is low	Some saliva samples contain very little DNA. This varies from individual to individual based on numerous variables.
DNA does not perform well in downstream applications.	DNA was not washed twice with Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed twice with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry step after DNA wash is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

Related Products	Product #
Saliva DNA Isolation Kit	RU45400
Saliva DNA Collection and Preservation Devices (50)	RU49000
Saliva DNA Collection, Preservation and Isolation Kit	RU35700
Buccal DNA Collection and Preservation Kit	33900

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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