3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) Product Insert

Product # RU62900

Norgen's Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) provides a fast and reproducible high-throughput method for isolating genomic DNA from saliva samples collected and preserved using Norgen's Saliva DNA Collection and Preservation Devices, as well as fresh saliva. 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 Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) can also be integrated with a robotic automation system.

Norgen's Purification Technology

Purification is based on the use of magnetic beads that bind DNA under optimized DNA binding conditions. 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 1 hour at 55°C. The samples are then transferred to the 96-Well Plate, and then Magnetic Bead Suspension and ethanol are added to the sample, followed by an incubation for 10 minutes at room temperature. The resulting solution is then placed on the magnetic separation rack. Only the DNA will bind to the magnetic beads, while most of the proteins will be removed in the supernatant. The bound DNA is then washed with 70% ethanol in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer B. The purified DNA can be used in a number of downstream applications.

Specifications

Kit Specifications	
Maximum Saliva Input	0.5 mL preserved saliva 0.25 mL fresh saliva
Average Yield from 0.25 mL of Saliva*	2 - 5 μg
Average Purity (OD260/280)	1.6 – 1.8
Time to Complete 96 Purifications	45 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. This kit is stable for 1 year after the date of shipment. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer. The buffered Proteinase K is stable for up to 1 year after the date of shipment when stored at room temperature.

Advantages

- Sample collection is non-invasive and painless
- Fast and easy processing using a magnetic bead system
- 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)
- High throughput and compatible with an automation robotic system

Kit Components

Component	Product #RU62900 (192 samples)
Lysis Buffer F	2 x 30 mL
Proteinase K in Storage Buffer	4 mL
Elution Buffer B	30 mL
Magnetic Bead Suspension	8.5 mL
96-Well Plate	2
96-Well Elution Plate	2
Adhesive Tape	2
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*.

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
- 96-well magnetic bead separation plate (i.e. V&P scientific, Inc. VP 771MDWM-1)
- Multi-channel micropipettors
- Microcentrifuge tube
- Water (for rinsing mouth)
- Norgen's Saliva DNA Collection and Preservation Devices (optional)
- Fresh 70% ethanol
- 96-100% ethanol
- 55°C Incubator
- (Optional) Benchtop centrifuge machine

Procedure

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).
- The protocol provided is for the 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, Magnetic Bead Suspension**, and **ethanol** used in Section 1 proportionally.
- **Always** vortex the Proteinase K before use.
- **Always** vortex the Magnetic Bead Suspension

Flow Chart

Procedure for Purifying Saliva DNA using
Norgen's Saliva DNA Isolation 96-Well Kit (Magnetic Bead System)

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 1 hour. Add Magnetic Bead Suspension and 96-100% ethanol. Mix and transfer to 96-Well Plate. Incubate for 10 minutes.

Place onto magnetic plate.
Let stand for 2 minutes.

Discard supernatant. Add 70% ethanol, mix and incubate for 1 minute.

Place onto magnetic plate.
Let stand for 2 minutes.

Discard supernatant. Repeat 70% ethanol wash a second time. Discard supernatant.

Place plate at 65˚C for 5 minutes to remove residual ethanol.

Add Elution Buffer B, mix and incubate at 65° or room Temperature for 5 minutes.

Place onto magnetic plate.
Let stand for 1 minute.

Carefully transfer supernatant to Elution Plate.

Pure Saliva DNA

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 by inversion for a few seconds.
c. Aliquot 500 µL of preserved saliva to a 2 mL microcentrifuge tube (not provided).
d. Add 20 µL of **Proteinase K** (vortex prior to use). Mix by vortexing and incubate at 55°C for 1 hour.
e. Add 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the saliva sample.
f. Add 900 µL of 96-100% ethanol and mix by gentle pipetting. Transfer to the 96-Well Plate (provided).
g. Incubate at room temperature for 10 minutes.
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 milliliters of water for 10 seconds in order to remove any food particles that may be present. If food particles are present they may interfere with the binding of the DNA to the magnetic beads.
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 2 mL microcentrifuge tube (not provided).
d. Add 250 µL **Lysis Buffer F**, and mix by vortexing or pipetting.
e. Add 20 µL of **Proteinase K** (vortex prior to use). Mix by vortexing and incubate at 55°C for 1 hour.
f. Add 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the saliva sample.
g. Add 900 µL of 96-100% ethanol and mix by gentle pipetting. Transfer to the 96-Well Plate (provided).
h. Incubate at room temperature for 10 minutes.
i. Proceed to Section 2: **Saliva DNA isolation**.

2. Saliva DNA Isolation

a. Place the 96-Well Plate on the 96-well magnetic plate and allow to sit for 2 minutes.
b. Leaving the 96-Well Plate on the magnetic plate, carefully discard supernatant by aspiration or pipetting without touching the magnetic beads.
c. Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of freshly prepared **70% ethanol**. Mix by pipetting and incubate at room temperature for 1 minute.
d. Place the 96-Well Plate on the magnetic plate and allow to sit for 2 minutes.
e. Leaving the 96-Well plate on the magnetic plate, carefully discard the supernatant by pipetting without touching the magnetic beads.
f. Repeat **Steps c-e** for a second wash step.

> **Note:** Remove as much of the 70% ethanol in the sample plate as possible by pipetting.

g. Place the 96-Well Plate at 65˚C for 5 minutes to remove any residual 70% ethanol.
h. Add 75 µL of **Elution Buffer B**. Mix by vortexing or gentle pipetting and incubate at 65°C for 5 minutes or in a laminate hood for 5 minutes.

> **Note:** Ensure that the magnetic beads at the bottom of the wells are fully resuspended with the Elution Buffer B.

i. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
j. Leaving the 96-Well plate on the magnetic plate, carefully transfer the elution to a 96-Well Elution Plate (provided) without touching the magnetic beads.
k. The purified DNA sample may be stored at 4°C for a few days. The provided adhesive tape can be used for the storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Magnetic beads were accidently pipetted up with the supernatant.	The pipette tip was placed too close to the magnetic beads while pipetting	Return the magnetic beads and the supernatant back into the sample well. Mix well, and place the plate back onto the magnetic separation plate for the specified time. Carefully remove the supernatant without touching the magnetic beads.
The yield of genomic DNA is low	Incomplete lysis of cells	Increased Proteinase K incubation time at 55ºC may result in increased yields.
	Incubation time with Magnetic Beads was not sufficient	Ensure that the Magnetic Bead Suspension is well mixed with the saliva and ethanol mix and incubate at room temperature for a minimum of 10 minutes (up to 20 minutes).
	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 70% ethanol	Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with 70% ethanol. Salt may interfere with downstream applications, and thus must be washed from the magnetic beads.
	Ethanol carryover	Ensure that any residual 70% ethanol is removed by pipetting after the drying step. 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 Isolation Kit (Magnetic Bead System)	RU55400
Saliva DNA Collection and Preservation Devices (50)	RU49000
Saliva DNA Collection, Preservation and Isolation Kit	RU35700
Saliva DNA Isolation Kit (50 Prep)	RU45400
Saliva DNA Isolation 96-Well Kit - 2 Plates (192 preps)	35200

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.

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

