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Saliva DNA Isolation Kit (Magnetic Bead System) – 50 Preps Product Insert

Product # RU55400

Norgen's Saliva DNA Isolation Kit (Magnetic Bead System) provides a fast and reproducible 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 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 10 minutes at 55°C, then Binding Buffer B and Magnetic Bead Suspension are added to the sample, followed by a second incubation for 5 minutes at 55°C. Isopropanol is then added to the mixture, and 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	
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 - 10 µg
Average Purity (OD260/280)	1.6 – 1.8
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. 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). These reagents should remain stable for at least 2 years in their unopened containers.

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)

Kit Components

Component	Product #RU55400 (50 samples)
Lysis Buffer F	30 mL
Proteinase K	1.2 mL
Binding Buffer B	12 mL
Magnetic Bead Suspension	2 x 1.1 mL
Elution Buffer B	8 mL
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
- Magnetic bead separation rack
- Micropipettors
- Water (for rinsing mouth)
- Norgen's Saliva DNA Collection and Preservation Devices (optional)
- 70% ethanol
- Isopropanol
- 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, Binding Buffer B, Magnetic Bead Suspension**, and **Isopropanol** 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 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 10 minutes. Add Binding Buffer B and Magnetic Bead Suspension. Mix and incubate at 55°C for 5 minutes. Add isopropanol, mix

Place tube in magnetic separation rack. Let stand for 2 minutes.

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

Place tube in magnetic separation rack. Let stand for 2 minutes.

Discard supernatant.

Incubate open tube at 65˚C for 3 minutes or 5 minutes at room temperature.

Add Elution Buffer B, mix and incubate for 1 minute.

Place tube in magnetic separation rack. Let stand for 2 minutes.

Carefully transfer supernatant to Elution Tube.

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 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** and 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.
g. Add 500 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 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 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** and 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the saliva sample. Mix by vortexing and incubate at 55°C for 5 minutes.
g. Add 500 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 magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 2 minutes. Occasionally invert the tube with the rack.
b. Leaving the tube in the rack, open the tube and carefully discard supernatant by pipetting without touching the magnetic beads.
c. Remove the sample tube from the magnetic rack and gently add 500 µL of **70% ethanol**. Mix by vortexing and incubate at room temperature for 1 minute.
d. Place the sample tube on the magnetic rack and allow to sit for 2 minutes. Occasionally invert the tube with the rack.
e. Leaving the tube in the rack, open the tube and carefully discard the supernatant by pipetting without touching the magnetic beads

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

f. Leaving the tube in the rack, gently place the open tube at 65˚C for 3 minutes or 5 minutes at room temperature to remove any residual 70% ethanol.
g. Remove the sample tube from the magnetic rack and add 75 µL of **Elution Buffer B**. Mix by vortexing and incubate at room temperature for 1 minute.
h. Place sample tube on the magnetic rack and allow to sit for 1 minute.
i. Leaving the tube in the rack, open the tube and carefully transfer the elution to a fresh 1.7 mL elution tube (provided), being careful not to touch the magnetic beads.

(Optional): Depending on the saliva sample, the elution may appear turbid. While this does not interfere with PCR amplification, the elution may be centrifuged at 14,000 x g (20,000 RPM) for 1 minute and the clear supernatant can be transferred to a new tube for a clean elution. This may reduce overall DNA yield.

j. 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
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 tube. Mix well, and place the tube back onto the magnetic separation rack 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.
	Magnetic beads added was not sufficient	Ensure that prior to use, the magnetic bead suspension has to be well mixed to avoid any inconsistency in DNA isolation.
	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 the drying step after the 70% ethanol 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
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.

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