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Microbiome DNA Isolation Kit — Product Insert

Product # 64100

Norgen's Microbiome DNA Isolation Kit provides a convenient and rapid method to isolate total DNA from a variety of Microbiome samples preserved using Norgen's Swab Collection & DNA Preservation Kit, as well as samples preserved using Norgen's other preservation devices and fresh swab samples. This universal protocol conveniently allows for the isolation of total genomic DNA from both host and microbial cells found in the swab sample simultaneously. The kit removes all traces of PCR impurities, and purifies the DNA using a simple and rapid spin column procedure. The purified DNA is of the highest quality and is fully compatible with downstream PCR or metagenomics using NGS.

Norgen's Purification Technology

Purification is based on spin column chromatography. The process provides a simple and convenient DNA isolation protocol for a variety of Microbiome samples preserved using Norgen's Swab Collection & DNA Preservation Kit. First, Lysis Additive A is added to the swab collection tube and incubated at 65˚C in order to efficiently and rapidly homogenize the sample. The sample is then centrifuged, and the supernatant is transferred to a DNase-free microcentrifuge tube. Binding Buffer I is added, and the lysate is incubated for 10 minutes on ice. The lysate is then spun for 2 minutes to pellet any cell debris, the supernatant is collected, an equal volume of 70% ethanol is added to the lysate and the solution is loaded onto a spin-column. Norgen's spin column binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the proteins and other contaminants are removed in the flowthrough or retained on top of the resin. The bound DNA is then washed using the provided Binding Buffer B and Wash Solution A, and the purified DNA is eluted using the Elution Buffer B. The purified total DNA is free of all inhibitors, including humic acid, and can be used in sensitive downstream applications including PCR.

Kit Components

Component	Product #64100 (50 preps)
Lysis Buffer E	55 mL
Lysis Additive A	6 mL
Binding Buffer I	7 mL
Binding Buffer B	30 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Specifications

Kit Specifications	
Maximum Sample Input	1 mL of preserved swab sample * or up to 0.5 mL preserved samples**
Swab Samples Tested	Fecal, Saliva, Buccal, Food, Nasal, Blood, Surface, Skin
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	700 µL
Time to Complete 10 Purifications	30 minutes

* Collected using Norgen's Swab DNA Collection & Preservation Kit (Cat. 45681)
** Please see the table in Section B - S*amples Collected using Norgen's Preservation Devices*

Advantages

- Universal method to detect microorganisms and host cells simultaneously in samples collected using a swab
- Rapid and convenient spin-column format
- Remove all PCR inhibitors from DNA samples
- Isolate high quality total DNA for PCR and metagenomics applications

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years from 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*.

Customer-Supplied Reagents and Equipment

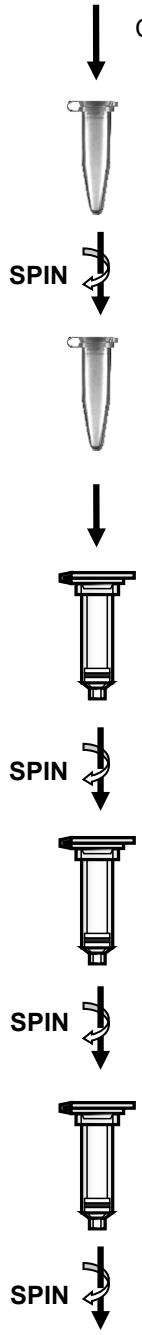
You must have the following in order to use the Microbiome DNA Isolation Kit:

- Benchtop microcenrifuge
- DNase-free microcentrifuge tubes
- Vortexer
- 96-100% ethanol
- 70% ethanol

Flow Chart

Procedure for Purifying Total DNA using Norgen's Microbiome DNA Isolation Kit

Add Lysis Buffer E and Lysis Additive A to sample as per Lysate Preparation instructions



Centrifuge. Transfer lysate.

Add Binding Buffer I.
Incubate for 10 minutes on ice.

SPIN

Transfer lysate

Add Ethanol

Bind to column

SPIN

Wash once with Binding Buffer B.
Wash twice with Wash Solution A.

SPIN

Elute DNA with
Elution Buffer B

SPIN

Purified Total DNA

Procedures

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

- All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of **Wash Solution A** by adding 42 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

1. Lysate Preparation

A. For Fresh Samples Collected using Swab

a. Add 1 mL of **Lysis Buffer E** to a tube containing the swab(s). Vortex briefly.
b. Add 100 µL of **Lysis Additive A** to the tube containing the swab(s) and vortex briefly.
c. Incubate the tube containing the swab(s) at 55˚C for 30 minutes and **Proceed to Step Cc**.

B. For Samples Collected using Norgen's Preservation Devices

a. Please refer the table below for the recommended input volume to proses. Transfer 0.2 – 0.5 mL to a 2 mL DNase-free microcentrifuge tube (not provided).

Sample	Kit or Devices	Cat. No.	Recommended Volume to Process
Saliva	Saliva DNA Collection and Preservation Devices	RU49000	0.5 mL preserved sample
Fecal	Fecal Swab DNA Collection & Preservation System	45670-B	0.2 mL preserved sample
	Fecal DNA Collection & Preservation Mini Tubes	27650	
	Stool Nucleic Acid Collection and Preservation Tubes	45630/45660	
	Stool Nucleid Acid Collection and Preservation System	63700	
Milk	Milk DNA Preservation and Isolation Kit	44800	0.5 mL preserved sample

b. Add **Lysis Buffer E** up to 1 mL and vortex. For example, add 0.8 mL of **Lysis Buffer E** to 0.2 mL of preserved fecal sample or 0.5 mL of **Lysis Buffer E** to 0.5 mL of preserved saliva or milk sample.
c. Add 100 µL of **Lysis Additive A** to lysis buffer and sample mixture and vortex briefly.

d. Incubate the lysis buffer and sample mixture at 65˚C for 5 minutes and **Proceed to Step Cd.**

C. For a Swab Sample Collected using Norgen's Swab Collection & DNA Preservation Kit (Cat. 45681).

a. Add 100 µL of **Lysis Additive A** to the swab collection tube and vortex briefly.
b. Incubate the swab collection tube at 65˚C for 5 minutes.
c. Carefully remove the swab from the collection tube.
d. Transfer up to 1 mL of the preserved sample to 2 mL DNAase-free microcentrifuge tube (not provided).
e. Centrifuge the tube for 2 minutes at **20,000 × g (~14,000 RPM)**. A thin white layer will form on the top of the supernatant.
f. Carefully transfer 700 µL of supernatant, without the white layer debris, to a DNase-free microcentrifuge tube (not provided).
g. Add 100 µL of **Binding Buffer I**, mix by inverting the tube a few times, and incubate for 10 minutes on ice.
h. Spin the lysate for 2 minutes at **20,000 x g (~14,000 RPM)** to pellet any cell debris.
i. Using a pipette, transfer up to 700 µL of supernatant (avoid contacting the pellet with the pipette tip) into a 2 mL DNase-free microcentrifuge tube (not provided).
j. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2.**

2. Binding to Column

a. Assemble a spin column with one of the provided collection tubes.
b. Apply 700 µL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **10,000 x g (~10,000 RPM)**. Discard the flowthrough and reassemble the spin column with the collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **20,000 x *g* (~14,000 RPM)**.

c. Repeat step **2b** with the remaining volume of lysate mixture.

3. Column Wash

a. Apply 500 µL of **Binding Buffer B** to the column and centrifuge for 1 minute at **10,000 x g (~10,000 RPM).**

Note: Ensure the entire Binding Buffer B 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. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 10,000 x g (~10,000 RPM).
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat **3c** and **3d**.
f. Spin the column for 2 minutes at **20,000 x g (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.

b. Add 50 µL of **Elution Buffer B** to the column.
c. Centrifuge for 1 minute at **425 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **20,000 x *g* (~14,000 RPM)**. If the entire volume has not been eluted, spin the column at **20,000 x g (~14,000 RPM)** for 1 additional minute.
d. **(Optional):** An additional elution may be performed if desired by repeating steps **4b** and **4c** using 50 µL of Elution Buffer. The total yield can be improved by an additional 20-30% when this second elution is performed.

5. Storage of DNA
The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	An alternative elution buffer was used	It is recommended that the Elution Buffer B supplied with this kit be used for maximum DNA recovery.
	Lysis Additive A was not added to the lysate	Ensure that the provided Lysis Additive A is added to increase DNA yield. Also, an incubation can be preformed at 65°C for 10 minutes after addition of the Lysis Additive A and prior to vortexing to maximize DNA recovery.
	Ethanol was not added to the lysate	Ensure that an equal amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 42 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
DNA does not perform well in downstream applications	Elution is brown	Ensure that the Lysis Additive A is added. Also ensure Binding Solution I is added to the lysate and that it is incubated on ice for 10 minutes prior to spinning down the lysate. Avoid any contact with the pellet or surface residue when collecting the supernatant after the 5 minute spin during Sample Preparation.
	Lysis Additive A was not added to the lysate	Ensure that the provided Lysis Additive A is added to the lysate.
	DNA was not washed with the provided Binding Buffer B and Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the provided Binding Buffer B and Wash Solution A. 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.
Problem	**Possible Cause**	**Solution and Explanation**

DNA does not perform well in downstream applications	Binding Buffer I was not added to the lysate	Ensure that the Binding Buffer I is added to the lysate and that it is incubated on ice for 10 minutes prior to spinning down the lysate.
	PCR reaction conditions need to be optimized	Take steps to optimize the PCR conditions being used, including varying the amount of template, changing the source of Taq polymerase, looking into the primer design and adjusting the annealing conditions.

Related Products	**Product #**
Swab Collection & DNA Preservation Kit	45680-B
Fecal Swab DNA Collection & Preservation System	45670-B
Milk Bacterial DNA Isolation Kit	21550
Saliva DNA Isolation Kit	RU45400
Saliva DNA Collection and Preservation Devices	RU49000
Fecal DNA Collection & Preservation Mini Tubes	27650
Stool Nucleic Acid Collection and Preservation Tubes	45630, 45660
Stool Nucleic Acid Collection and Preservation System	63700
Bacterial Genomic DNA Isolation Kit	17900, 17950
Stool DNA Isolation Kit	27600

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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