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

Product # 63100

Norgen's Stool DNA Isolation 96-Well Kit (Magnetic Bead System) provides a fast and reproducible high–throughput method for isolating genomic DNA from stool samples collected and preserved using Norgen's Stool Nucleic Acid Collection and Preservation Tubes, as well as fresh or frozen stool. Stool DNA purified using Norgen's kit is of the highest quality and is compatible with a number of downstream research applications including PCR, NGS sequencing and microarray analysis. Norgen's Stool 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 binding conditions. Stool DNA can be isolated from stool samples collected and preserved using Norgen's Stool Nucleic Acid Collection and Preservation Tubes, as well as fresh or frozen stool samples. The stool samples are first mixed with Lysis Buffer L and Lysis Additive A in the provided bead tube and homogenized. The clean lysate is then separated by centrifugation, followed by the addition of Binding Buffer I and incubation on ice for 10 minutes. The lysate is then spun in order to remove any debris and the supernatant is then transferred into a well of the 96-well plate. Magnetic Bead Suspension and ethanol are then added to the clean supernatant and the resulting solution is placed on the magnetic separation rack. Only the DNA will bind to the magnetic beads, while most of the proteins will be in the supernatant that is discarded. The bound DNA is then washed with 70% ethanol in order to remove any remaining impurities and the purified total DNA is eluted with Elution Buffer B. The purified DNA can be used in a number of downstream applications.

Specifications

Kit Specifications	
Maximum Stool Input	0.2 mL preserved stool 0.2 g fresh or frozen stool
Average Yield from 0.25 mL of Stool*	15 - 25 μg
Average Purity (OD260/280)	1.7 – 1.8
Time to Complete 96 Purifications	50 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.

Advantages

- Fast and easy processing using a magnetic bead system
- Robust lysis system (chemical lysis combined with a mechanical homogenization)
- Isolate high quality genomic DNA
- Compatible with preserved stool samples collected using Norgen's Stool Nucleic Acid Collection and Preservation Tubes (please see Related Products Table)
- High yields - Consistent, high yields of inhibitor-free DNA up to 50 kb plus
- High throughput and compatible with an automation robotic system

Kit Components

Component	Product # 63100 (192 samples)
Lysis Buffer L	3 x 60 mL 1 x 30 mL
Lysis Additive A	25 mL
Binding Buffer I	25 mL
Solution WN	55 mL
Elution Buffer B	30 mL
Magnetic Bead Suspension	4 x 1.1 mL
Bead Tubes	196
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*.

Solution WN 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. Stool 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 stool.

Customer-Supplied Reagents and Equipment

- Magnetic bead separation plate
- Multi-channel micropipettors
- Microcentrifuge tube
- Flat bed vortex or bead beater equipment(e.g. MP Biomedicals' FastPrep®-24 Instrument)
- Norgen's Stool Nucleic Acid Collection and Preservation Tubes (optional)
- 70% ethanol (prepare fresh)
- 96-100% ethanol
- Ice

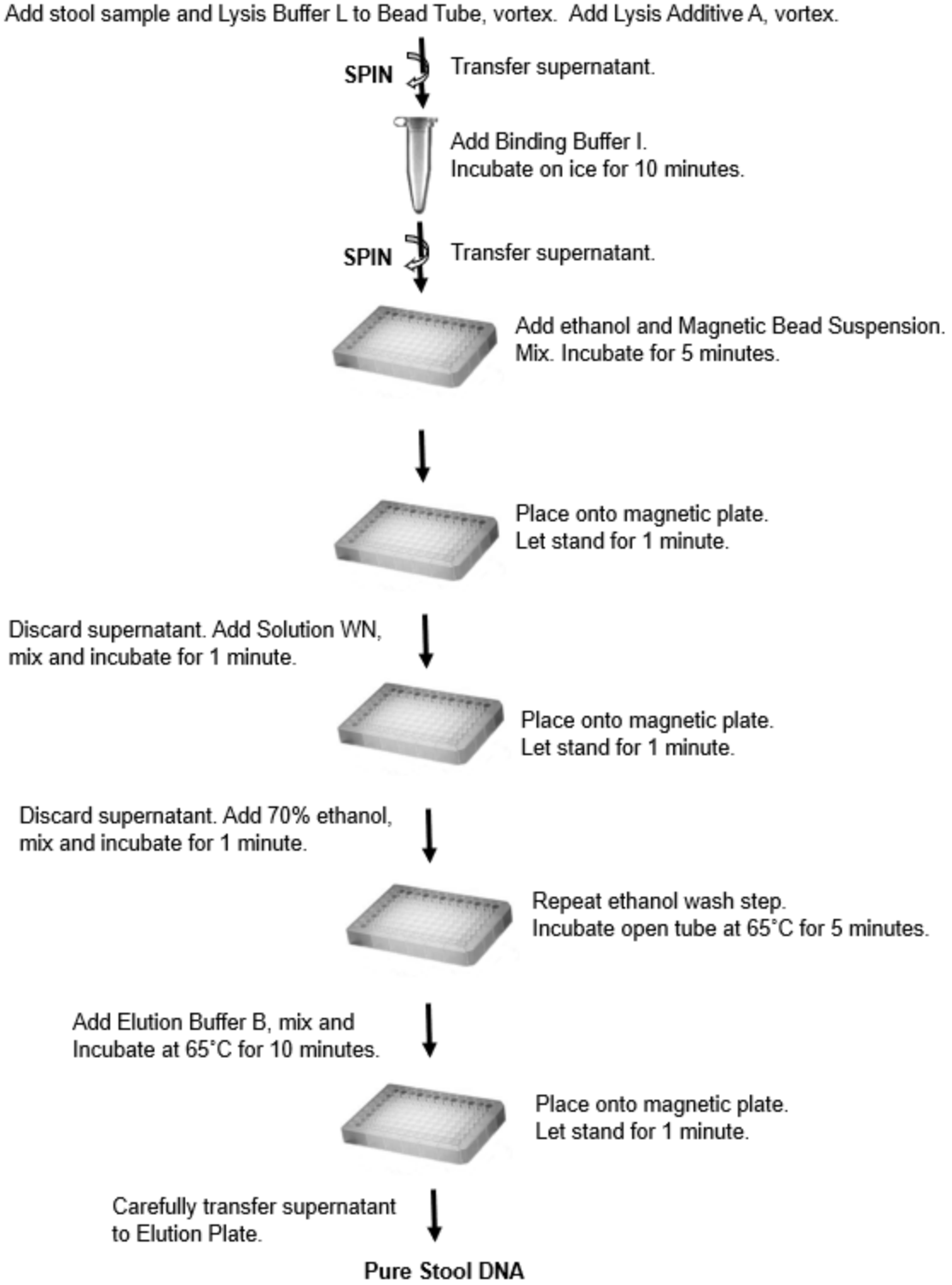
Procedure

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.
- Optionally, stool samples can be collected and preserved using Norgen's Stool Nucleic Acid Collection and Preservation Tubes (please see Related Products Table).
- **Always** vortex the **Magnetic Bead Suspension** before use.
- Prepare a working concentration of the **Solution WN** by adding 73 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Solution WN**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Flow Chart

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



1. Stool Sample Collection and Lysate Preparation

a. Add up to 200 mg of fresh or frozen stool sample to a provided Bead Tube and add 1 mL of Lysis Buffer L. Vortex briefly to mix stool and **Lysis Buffer L**.

For stool samples that have been preserved using Norgen's **Stool Nucleic Acid Collection and Preservation Tubes (Cat# 45650)**, add 200 µL of preserved sample to a provided Bead Tube and add 800 µL of **Lysis Buffer L**. Vortex briefly to mix stool and Lysis Solution.

b. Add 100 µL of **Lysis Additive A** and vortex briefly.
c. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. Scientific Industries' Disruptor Genie TM). Vortex for 3 minutes at maximum speed.
d. Centrifuge the tube for 2 minutes at 20,800 × g (~14,000 RPM).
e. Transfer up to 600 µL of supernatant to a DNase-free microcentrifuge tube (not provided).
f. Add 100 µL of **Binding Buffer I**, mix by inverting the tube a few times, and incubate for 10 minutes on ice.
g. Spin the lysate for 2 minutes at 20,800 x g (~14,000 RPM) to pellet any cell debris.
h. Using a pipette, transfer up to 600 µL of supernatant (avoid contacting the pellet with the pipette tip) into a 96-Well Plate.
i. Add 300 µL of 96-100% ethanol (provided by the user) and 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by gently pipetting.
j. Incubate at room temperature for 5 minutes with shaking.
k. Proceed to Section 2: Stool DNA isolation.

2. Stool DNA Isolation

a. Put the 96-Well Plate on the magnetic plate (not provided). Allow to sit for 1 minute.
b. Aspirate and discard supernatant without touching the magnetic beads.
c. Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of **Solution WN** (ensure ethanol was added). Resuspend by pipetting and incubate at room temperature for 1 minute with continuous shaking.
d. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
e. Aspirate and discard supernatant without touching the magnetic beads.
f. Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of freshly prepared **70% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute with continuous shaking.
g. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
h. Aspirate and discard supernatant without touching the magnetic beads.
i. Repeat **Steps 2f - 2h** for a second wash step.

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

j. Incubate the 96-Well Plate at 65˚C for 5 minutes to dry the magnetic beads.
k. Add 75 -100 µL of **Elution Buffer B**. Mix by pipetting and incubate at 65˚C for 10 minutes with shaking.
l. Briefly mix and place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
m. Carefully transfer the elution to a 96-Well Elution Plate (provided) without touching the magnetic beads. 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.

Related Products	Product #
Stool DNA Isolation Kit (Magnetic Bead System)	55700
Stool Nucleic Acid Collection and Preservation Tubes	45630, 45660
Stool DNA Isolation Kit (50 Prep)	27600
Stool Nucleic Acid Isolation Kit	45600
Stool Nucleic Acid Collection and Preservation System	63700

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	Ensure that Lysis Additive A is added. Also incubation at 65ºC may result in increased yields.
	Amount of magnetic beads added was not sufficient	Ensure that the magnetic bead suspension is mixed well prior to use to avoid any inconsistency in DNA isolation.
	DNA concentration in the stool sample being used is low.	Some stool samples contain very little target DNA. This varies from individual to individual based on numerous variables. Incubation at 65ºC 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.

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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