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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 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. Using this kit, DNA can be extracted either manually or by using automation platform IsoPure™. The protocol can also be easily modified by making minor changes to be able to run on other Magnetic Bead-based automation platforms such as KingFisher™ Flex 96 and Hamilton MagEx STAR platforms.

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. The stool samples are first mixed with Lysis Buffer L and Lysis Additive A in the provided Bead B Tube and homogenized. The clean lysate is then separated by centrifugation, followed by the addition of Binding Buffer I and incubation on ice for 5 minutes. Magnetic Beads B and ethanol are then added to the clean supernatant and the resulting solution is placed on the magnetic separation plate. 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 Solution WN and 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	
Number of Preps	192
Maximum Stool Input	0.2 mL preserved stool 0.2 g fresh or frozen stool
Average Yield from 0.25 mL of Stool*	4 - 25 µg
Average Purity (OD260/280)	1.7 – 1.9
Time to Complete 96 Purifications (automated)	40 minutes (hands-on time)
Time to Complete 96 Purifications (manual)	90 minutes (hands-on time)

* 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.

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 Safety Data Sheets (SDSs). 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 from 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.

Kit Components

Component	Product # 63100 (192 samples)
Lysis Buffer L	3 x 60 mL 1 x 30 mL
Lysis Additive A	25 mL
Magnetic Beads B	2 x 2.2 mL
Solution WN	55 mL
Binding Buffer I	25 mL
Elution Buffer B	30 mL
Bead B Tubes	200
96-Well Plate	2
96-Well Elution Plate	2
Adhesive Tape	2
Product Insert	1

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 Stool Nucleic Acid Collection and Preservation Tubes (please see Related Products Table)
- High yields - Consistent, high yields of inhibitor-free DNA
- Magnetic Bead protocol can easily be adapted on a variety of automation systems (IsoPure™, KingFisher, Hamilton Star/Vantage, and Tecan)
- Purified DNA is suitable for a variety of downstream applications, including qPCR or NGS sequencing.

Customer-Supplied Reagents and Equipment

- Magnetic Bead 96-Well Separation Plate (For manual isolation)
- Adhesive Tape (For manual isolation) – We recommend Norgen Cat. 28394
- IsoPure™ 96 or IsoPure™ Mini Purification System (For automated isolation)
- Micropipettors
- Microcentrifuge tube
- Norgen's Stool Nucleic Acid Collection and Preservation Tubes (optional)
- Flat bed vortex or bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 Instrument)
- 70% ethanol (prepare fresh)
- 96-100% ethanol
- Temperature adjustable (37°C, 65ºC) incubator(s)
- Orbital Shaker
- RNase A (optional)
- Ice

Procedure

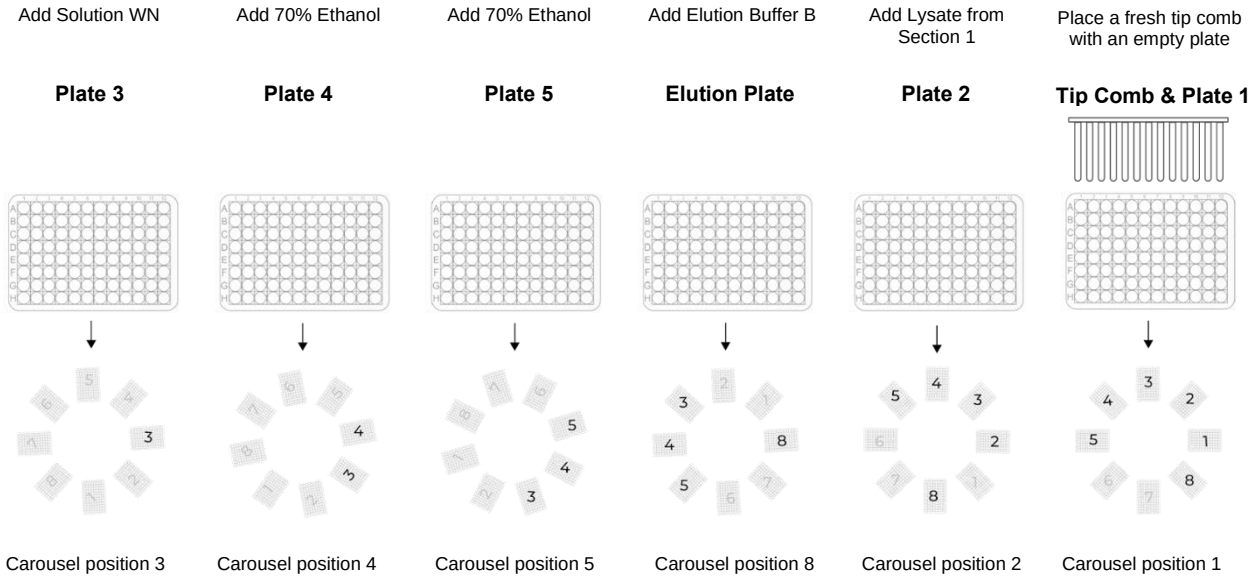
Notes prior to use:

- The procedure provides information and steps to follow to extract DNA using automated as well as manual methods.
- **Section 1** describes the procedure to prepare lysate while **Section 2** describes automated and manual purification of DNA from the prepared lysate.
- 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.

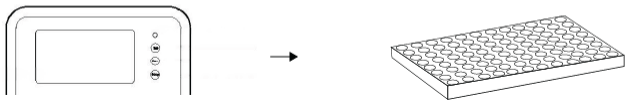
- Optionally, stool samples can be collected and preserved using Norgen's Stool Nucleic Acid Collection and Preservation Tubes (please see Related Products Table).
- **Always** vortex the **Magnetic Beads B** before use.
- Preheat the incubator(s) according to the temperatures required (37°C or 65ºC). For automation procedures, the automation platform that is being used may have a heat block to provide appropriate temperatures for incubation and thus an incubator might not be required. IsoPure™ instruments come with heat blocks and the scripts provided include the incubation at required temperature.
- Prepare a working concentration of the **Solution WN** by adding 73 mL of 96 - 100 % ethanol (not provided) 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 using Norgen's Stool DNA Isolation Kit (Magnetic Bead System) – IsoPure™ 96



Run Instrument

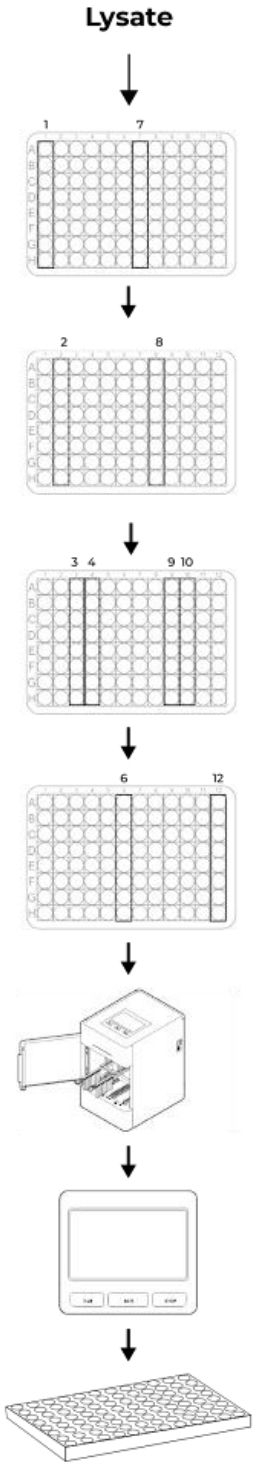


Place Plate 2 at position 2 and Run Program "STLDNA96"

Run Complete. Take Elution plate and store DNA.

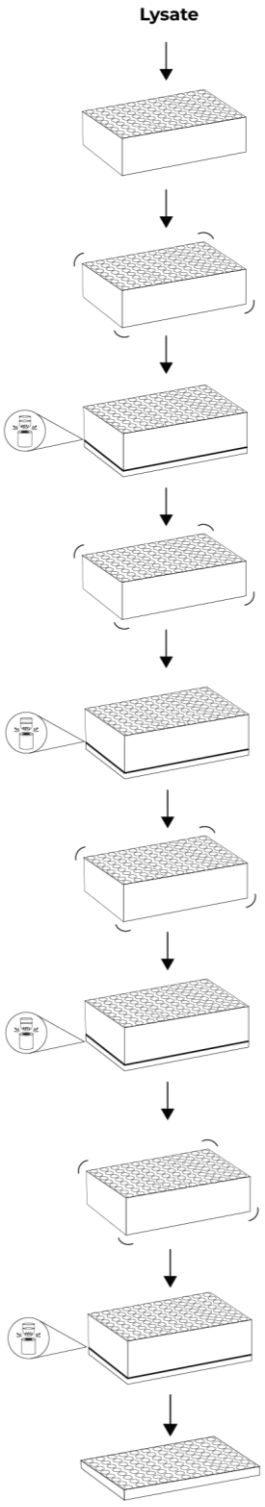
Flow Chart

Procedure for using Norgen's Stool DNA Isolation Kit (Magnetic Bead System) – IsoPure™ Mini



Flow Chart

Procedure for Norgen's Stool Isolation 96-Well Kit (Magnetic Bead System) - Manual Method



Prepare Lysate as per Section 1 Step i.
Transfer to 96-Well plate.

Add Ethanol and Magnetic Beads B and Mix.

Place Plate on magnetic plate.
Discard supernatant.

Add Solution WN and Mix.

Place Plate on magnetic plate.
Discard supernatant.

Add 70% Ethanol and Mix.

Place Plate on magnetic plate.
Discard supernatant.
Repeat the 70% Ethanol Wash.

Add Elution Buffer B. Incubate at 65°C.

Place Plate on magnetic plate.

Transfer DNA to a fresh Plate.

Stool DNA Isolation using Automation and Manual Method

This section outlines step-by-step instructions for Stool DNA extraction using both automated and manual methods. The automation method provides guidance for using IsoPure™ instruments – IsoPure™ 96 (Cat. AP1096) and IsoPure™ Mini (Cat. AP1016), however this protocol can also be easily adapted for other magnetic bead-based automation platforms, such as KingFisher™ Flex 96 and Hamilton MagEx STAR platforms.

Section 1 details the preparation of lysates from stool sample, applicable to both automated and manual DNA extraction methods.

Section 2 is divided into multiple subsections, offering detailed instructions for extracting DNA from the lysate prepared in **Section 1**, using either automated systems or manual procedures.

To prepare for Stool DNA extraction using automated systems, please follow the steps outlined below.

Set-up the IsoPure™ Plates

Notes Prior to Use

- Only use plates that are compatible with the instrument that is being used. Use 96 deep well plates for IsoPure 96 (Cat. AP1096-DWP), 96 well elution plates for IsoPure™ (Cat. AP1096-ELP) and Tip Comb for IsoPure 96 (Cat. AP1096-TC) while working with IsoPure™ 96 Instruments. Use 96 deep well plates for IsoPure 96 (Cat. AP1096-DWP) and 8-place magnetic tip comb for IsoPure™ Mini (Cat. AP1016-MC) while working with IsoPure™ Mini Instruments.
- The numbering of plates is based on where it should be placed on the carousel in the instrument. Use the carousel rotation keys on the instrument or select the carousel position on the screen to bring it to the right position as mentioned in the flow chart.
- IsoPure™ 96 uses multiple plates for DNA extraction while IsoPure™ Mini uses single plate for DNA extraction.
- For IsoPure™ 96 most of the lysate preparation is performed in plate 2 while other plates are used for performing washing and elution steps.
- For IsoPure™ Mini the lysate preparation is performed in column 1 and/or 7 while washing and elution steps could be performed in other columns of the same plate.
- For IsoPure™ 96, DNA elution step is always performed in plate 8.
- Prepare the plates and label them based on the sample type as mentioned in the tables below.

Table 1. Set-up for Stool DNA Extraction

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2.**

Component	IsoPure™ Mini	IsoPure™ 96	Location on Carousel	Amount
Tip Comb	In the instrument	Plate 1	1	Empty Plate
Lysate	Column 1 / Column 7	Plate 2	2	920 µL
Solution WN	Column 2 / Column 8	Plate 3	3	600 µL
70% ethanol	Column 3 / Column 9 and Column 4 / Column 10	Plate 4 & Plate 5	4 & 5	600 µL
Elution Buffer B	Column 6 / Column 12	Elution Plate	8	100 µL

Section 1. Stool Sample Collection and Lysate Preparation

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

   Note: For stool samples that have been preserved using Norgen's **Stool Nucleic Acid Collection and Preservation Tubes (Cat# 45660)**, add 200 µL of preserved sample to a provided Bead B 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 bead 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 fresh 96-well plate (not provided) or 1.7 mL DNase-free microcentrifuge tube (not provided).
f. Add 100 µL of **Binding Buffer I** to each sample.
g. Seal the plate with an Adhesive Tape (not provided) and mix the 96-well plate or tube by inverting a few times and incubate for 5 minutes on ice or at -20°C.
h. Spin the 96-well plate for 2 minutes at 3220 × g (~4,000 RPM) to pellet any debris (If using tubes, spin them at 20,000 × g (~14,000 RPM) for 2 minutes).
i. Transfer up to 600 µL of supernatant (avoid contacting the pellet with the pipette tip) into **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or **96-Well Plate** (Manual extraction).
j. Prepare a mixture of 300 µL of **96-100% ethanol** (not provided) and 20 µL of **Magnetic Beads B** (vortex prior to use) and add it to the lysate collected above. We recommend a 10% overage while making mixture for multiple samples, to compensate for pipetting errors. Make sure to vortex the bulk mix before adding it to the lysate. It is recommended to prepare the bulk fresh every time a new DNA isolation is performed.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of **RNase A** (not provided; make sure not to exceed 20 µL) to the lysate with **96-100% ethanol** and **Magnetic Beads B** mixture to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or DNase free **microcentrifuge tube** (not provided) for manual extraction during this step. If RNase treatment is performed, make sure to run program **STLDNA96R** (IsoPure™ 96) program instead of **STLDNA96** and **STLDNA16R** (IsoPure™ 16) program instead of **STLDNA16** to include an incubation step of 37°C for 15 minutes.

k. Proceed to Section 2: **DNA Isolation from Stool Lysate.**

Section 2: DNA Isolation from Stool Lysate

2.1 DNA Isolation using IsoPure™ 96 System

a. Setup plates according to **Table 1.** Add 600 µL **Solution WN** (added with ethanol) to **plate 3** and keep it at **position 3**.
b. Add 600 µL **70% ethanol** to **plate 4 & 5** and keep it at **position 4 & 5** respectively.
c. Add 100 µL of **Elution Buffer B** to the **Elution plate** and place it at **position 8** on the carousel.
d. Place sample plate **(Plate 2)** in the machine at **position 2**.
e. Place a clean tip comb in an empty plate **(Plate 1)** and keep it at **position 1.**
f. After setting up, run the program **STLDNA96** or **STLDNA96R**.
g. The instrument will prompt after the procedure is complete. Remove the elution plate from the instrument and the DNA is now ready for further downstream processing.
h. The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the **Elution Plate** for storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage.

2.2 DNA Isolation using IsoPure™ Mini System

a) Add 600 µL **Solution WN** (added with ethanol) to **Column 2 and/or Column 8**.
b) Add 600 µL 70% ethanol to **Column 3 and/or Column 9** as well as **Column 4 and/or Column 10**.
c) Add 100 µL of **Elution Buffer B** to **Column 6 and/or 12**.
d) Place a clean tip comb into IsoPure™ Mini.
e) After setting up, place the plate into the instrument and run the program **STLDNA16** or **STLDNA16R**.
f) The instrument will prompt after the procedure is complete. Remove the plate from the instrument and the DNA is now ready for further downstream processing.
g) Transfer the eluted DNA from **Column 6 and/or Column 12** to a fresh plate. The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the plate for storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage.

2.3 DNA Isolation using Manual Method

a) Seal the plate with an Adhesive Tape (not provided) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
b) Place the 96-Well Plate on the magnetic plate. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
c) Remove the Adhesive Tape. Aspirate and discard supernatant without touching the magnetic beads.
d) Remove the 96-Well Plate from the magnetic plate and gently add 600 µL mL of **Solution WN (ensure ethanol was added).**
e) Seal the plate with an Adhesive Tape (not provided) and mix the plate on an orbital shaker at 1000 rpm for 1 minute. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minute.
f) Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
g) Aspirate and discard supernatant without touching the magnetic beads.
h) Remove the 96-Well Plate from the magnetic plate and gently add 600 µL of freshly prepared **70% ethanol**.
i) Seal the plate with an Adhesive Tape (not provided) and mix the plate on an orbital shaker at 1000 rpm for 1 minute. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minute.
j) Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
k) Aspirate and discard supernatant without touching the magnetic beads.
l) Repeat **Steps h – k from this section** for a second wash step.

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

m) Incubate the 96-Well plate at room temperature for 5 to 10 minutes to air-dry the magnetic beads. Make sure not to over-dry the magnetic beads as this might affect the DNA yield.
n) Remove the sample plate from the magnetic plate and add 100 µL of **Elution Buffer B**. Mix by gently pipetting and incubate at 65˚C for 10 minutes.
o) Briefly mix by pipetting and place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
p) Carefully transfer the elution to a **96-Well Elution Plate** (provided) without touching the magnetic beads.
q) The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the **96-Well Elution Plate** for 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	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 Beads B 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.

Related Products	Product #
Stool DNA Isolation Kit (Magnetic Bead System)	55700
Stool Nucleic Acid Collection and Preservation Tubes	45660
Stool DNA Isolation 96-Well Kit (High Throughput) - (2x96-Well)	65600
Stool Nucleic Acid Isolation Kit	45600
Stool Nucleic Acid Collection and Preservation System	63700

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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