

**Stool DNA Isolation Kit (Magnetic Bead System) – 50 Preps**  
**Product # 55700**
**Product Insert**

Norgen's Stool DNA Isolation 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 Transport 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 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 Transport 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 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 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 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 Stool Input                  | 0.2 mL preserved stool<br>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 10 Purifications    | 35 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 (15 – 25°C). These reagents should remain stable for at least 2 years in their unopened containers.

**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 Transport Tubes (please see Related Products Table)
- High yields - Consistent, high yields of inhibitor-free DNA up to 50 kb plus

## Kit Components

| Component                | Product # 55700 (50 samples) |
|--------------------------|------------------------------|
| Lysis Buffer L           | 60 mL                        |
| Lysis Additive A         | 6 mL                         |
| Binding Buffer I         | 7 mL                         |
| Magnetic Bead Suspension | 1.1 mL                       |
| Solution WN              | 18 mL                        |
| Elution Buffer B         | 8 mL                         |
| Elution tubes (1.7 mL)   | 50                           |
| Bead tubes               | 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](http://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 rack
- Micropipettors
- Microcentrifuge tube
- Norgen's Stool Nucleic Acid Collection and Transport 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 Transport Tubes (please see Related Products Table).
- **Always** vortex the **Magnetic Bead Suspension** before use.
- Prepare a working concentration of the **Solution WN** by adding 24 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 42 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 Kit (Magnetic Bead System)

Add stool sample and Lysis Buffer L to Bead Tube, vortex. Add Lysis Additive A, vortex.

**SPIN**



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

**SPIN**



Transfer supernatant. Add ethanol and  
Magnetic Bead Suspension. Mix.  
Incubate for 5 minutes.



Place tube in magnetic separation rack.  
Let stand for 1 minute.

Discard supernatant. Add Solution WN,  
mix and incubate for 1 minute.



Place tube in magnetic separation rack.  
Let stand for 1 minute.

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



Repeat ethanol wash step.  
Incubate open tube at 65°C for 5 minutes.

Add Elution Buffer B, mix and  
Incubate at 65°C for 10 minutes.



Place tube in magnetic separation rack.  
Let stand for 1 minute.

Carefully transfer supernatant  
to Elution Tube.



**Pure Stool DNA**

## 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 Transport 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™). Vortex for 3 minutes at maximum speed.
- d. Centrifuge the tube for 2 minutes at 14000 × g (~14,000 RPM).
- e. Transfer up to 600 µL of supernatant to a DNAase-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 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 2 mL DNase-free microcentrifuge tube (not provided).
- i. Add 300 µL 96-100% ethanol (provided by the user) and 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above.
- j. Incubate at room temperature for 5 minutes. Occasionally invert the tube.
- k. Proceed to Section 2: Stool DNA isolation

## 2. Stool DNA Isolation

- a. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 1 minute.
- b. Aspirate and discard supernatant without touching the magnetic beads.
- c. Remove the sample tube from the magnetic rack and gently add 500 µL of **Solution WN** (ensure ethanol was added). Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
- d. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
- e. Aspirate and discard supernatant without touching the magnetic beads.
- f. Remove the sample tube from the magnetic rack and gently add 500 µL of freshly prepared **70% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
- g. Place the sample tube on the magnetic rack 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 tube as possible by pipetting.

- j. Incubate the open tube at 65°C for 5 minutes to dry the magnetic beads.
- k. Remove the sample tube from the magnetic rack and add 75 -100 µL of **Elution Buffer B**. Mix by vortexing and incubate at 65°C for 10 minutes.
- l. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 1 minute.
- m. Carefully transfer the elution to a fresh 1.7 mL elution tube (provided) without touching the magnetic beads. 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 accidentally 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  | Ensure that Lysis Additive A is added. Also incubation at 65°C 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 65°C 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 Nucleic Acid Collection and Transport Tubes | 45630, 45660 |
| Stool DNA Isolation Kit (50 Prep)                 | 27600        |
| Stool Nucleic Acid Isolation Kit                  | 45600        |

### 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.norgenbiotech.com](http://www.norgenbiotech.com)) or through email at [techsupport@norgenbiotech.com](mailto:techsupport@norgenbiotech.com).

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