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Stool DNA Isolation Kit Dx

Product Insert

REF **Dx27600** CE IVD PIDx27600-7

Intended Use

Norgen's Stool DNA Isolation Kit Dx provides a convenient and rapid method to isolate total DNA from fresh or frozen stool samples for subsequent *in vitro* diagnostic use. Purification is based on spin column chromatography. The universal protocol conveniently allows for the isolation of total genomic DNA from all the various microorganisms and host cells found in the stool sample simultaneously. The kit removes all traces of humic acid using the provided Bead Tubes and a combination of chemical and physical homogenization and lysis, without the use of phenol-chloroform extractions.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated using the DNA isolated with Norgen's Stool DNA Isolation Kit Dx in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's Stool DNA Isolation Kit Dx is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques including DNA isolation.

Norgen's Stool DNA Isolation Kit Dx does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Kit Components

Component	Product #Dx27600 (50 samples)
Lysis Solution	60 mL
Lysis Additive	6 mL
Binding Solution	7 mL
Wash Solution I	30 mL
Wash Solution II	22 mL
Elution Buffer	3 mL x 2
Bead Tube	50
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Label Legend

Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manu-facturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temper-ature limitation

Advantages

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Fits into *in vitro* diagnostic workflows
- Fast and easy processing using a rapid spin-column format
- No phenol:chloroform extractions
- Remove all humic acid from DNA samples
- Isolate high quality genomic DNA

Specifications

Kit Specifications	
Maximum Stool Input	200 mg fresh or frozen stool
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Time to Complete 10 Purifications	30 minutes

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

Precautions
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*.

Wash Solution I 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

- Benchtop microcenrifuge
- DNAse-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment
- 96-100% ethanol
- 70% ethanol

Procedure

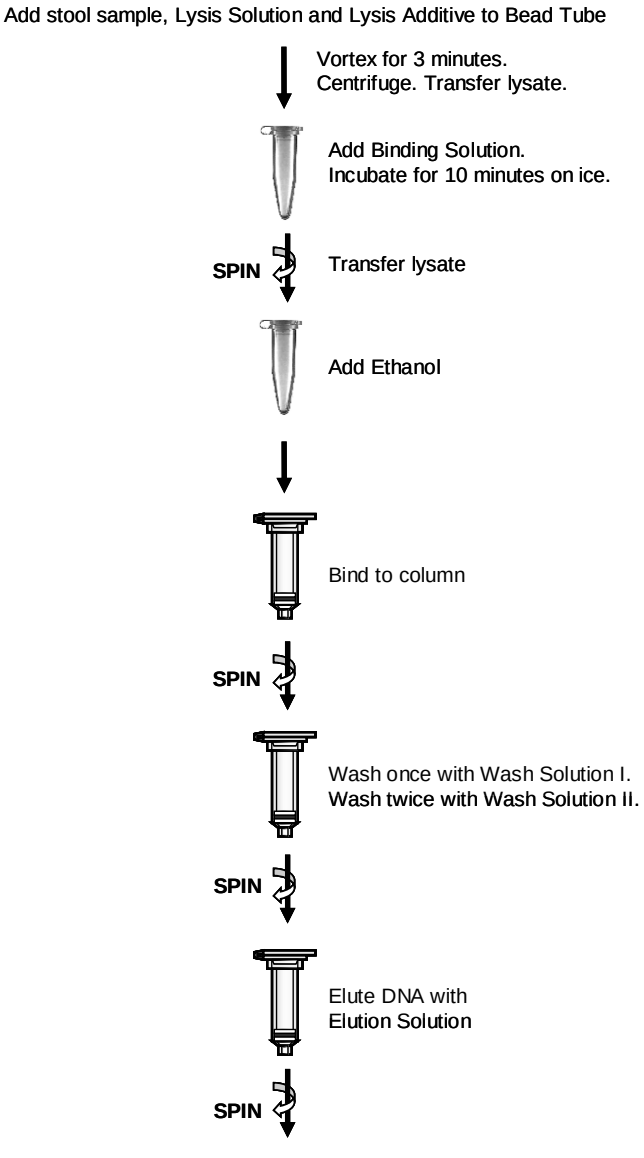
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can 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.

Flow Chart

Procedure for Purifying Stool DNA using Norgen's Stool DNA Isolation Kit Dx



Notes Prior to Use

- 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 II** by adding 50 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution II**. This will give a final volume of 72 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. Add up to 200 mg of stool sample to a provided Bead Tube and add 1 mL of **Lysis Solution**. Vortex briefly to mix stool and Lysis Solution.
b. Add 100 µL of Lysis Additive 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. OMNI BEAD RUPTOR). Vortex for 5 minutes at maximum speed for a flat-bed vortexer or S=5.00, T=0:20, D=0:10 and C=0.2 program on OMNI BEAD RUPTOR.
d. Centrifuge the tube for 2 minutes at **20,000 × 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 Solution, 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 500 µL of supernatant (avoid contacting the pellet with the pipette tip) into a 2 mL DNase-free microcentrifuge tube (not provided).
i. 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 600 µL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **20,000 × g (~14,000 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.

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

3. Column Wash

a. Apply 500 µL of **Wash Solution I** to the column and centrifuge for 1 minute at **10,000 × g (~10,000 RPM).**
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 500 µL of **Wash Solution II** to the column and centrifuge for 1 minute at **10,000 × 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 **10,000 × g (~10,000 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 100 µL of **Elution Buffer** to the column and incubate at room temperature for 1 minute.
c. Centrifuge for 1 minute at **10,000 x g (~10,000 RPM)**.

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	Homogenization was incomplete	Depending on the type of stool, further vortexing with the flat bed vortex or bead beater equipment may be required. However, it is not recommended to increase the vortex time to longer than 5 minutes at maximum speed. Also, ensure that the maximum input of 200 mg of stool is not exceeded, as this may also cause incomplete homogenization.
	An alternative elution buffer was used	It is recommended that the Elution Buffer supplied with this kit be used for maximum DNA recovery.
	Lysis Additive was not added to the lysate	Ensure that the provided Lysis Additive is added to separate humic acid and increase DNA yield. Also, an incubation can be performed at 65°C for 10 minutes after addition of the Lysis Additive 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 50 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
Poor DNA Recovery	Ethanol was not added to the Wash Solution II	Ensure that 50 mL of 96 - 100% ethanol is added to the supplied Wash Solution II prior to use.

DNA does not perform well in downstream applications	Eluted DNA sample is brown	Ensure that the Lysis Additive is added. Also ensure Binding Solution 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 was not added to the lysate	Ensure that the provided Lysis Additive is added to the lysate.
	DNA was not washed three times with the provided Wash Solutions	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the provided Wash Solutions. 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.
DNA does not perform well in downstream applications	Binding Solution was not added to the lysate	Ensure that the Binding Solution 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.

Product Use Restriction

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The respective user is liable for any and all damages resulting from application of Norgen's Stool DNA Isolation Kit Dx for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

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 support@norgenbiotek.com.

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