

E-Sphere™ Stool DNA Extraction Kit User Guide

Version 1.0

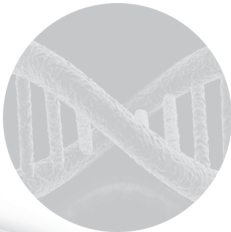
A rapid, easy-to-use method to purify genomic DNA from human stool samples.

REF Catalog Number E001001

50 DNA Extractions



Store at 4 °C



The E-Sphere™ Stool DNA Extraction Kit is intended for general laboratory use. This product is not intended to provide information for the diagnosis, prevention, or treatment of disease.




Symbol Glossary	
	Temperature limitation
	Caution, consult accompanying documents
	Catalog number

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E-Sphere™ Stool DNA Extraction Kit Catalog Number E001001

Box Contents

50 DNA Extractions

Component Name	Part Number	Count
E-Sphere™ Stool Buffer	E001001-001	1
E-Sphere™ Stool Enzyme	E001001-002	1
E-Sphere™ Stool Clean-Up Columns	E001001-003	50
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Storage/Stability



The E-Sphere™ Stool DNA Extraction Kit can be stored at 4 °C for up to 1 year.

Materials Not Supplied

● Required Equipment and Supplies

- ◆ 1.5-ml microcentrifuge tubes
- ◆ Laboratory pipettors capable of handling 5-1000 µl volumes
- ◆ Nuclease-free aerosol barrier pipette tips
- ◆ Microcentrifuge with rotor for 1.5-ml tubes capable of generating up to 8,000 x g
- ◆ One laboratory heat block or water bath capable of heating to 75 °C
- ◆ Laboratory vortex mixer
- ◆ Laboratory timer

● Optional Equipment and Supplies

- ◆ Laboratory thermal cycler
- ◆ Multiple sample attachment for vortex mixer



Safety Procedures

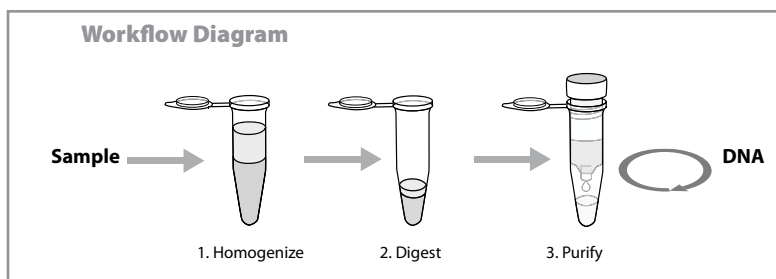
Always wear a lab coat, safety glasses, and disposable gloves when using the E-Sphere™ Stool DNA Extraction Kit. Dispose of all biohazardous materials according to your institutional biosafety procedures. For more information, refer to the appropriate Material Safety Data Sheet (MSDS) available online at www.phthisisdiagnostics.com

Product Application

The E-Sphere™ Stool DNA Extraction Kit allows users to quickly and easily extract total DNA from human stool samples. The purified DNA is of high quality and is suitable for downstream PCR analysis. The extraction procedure requires only 9 steps, uses standard molecular biology laboratory equipment, and can be completed in less than 30 minutes.

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Instructions for Use



Before Starting

Set the laboratory heat block or water bath to 75 °C and allow sufficient time for the device to equilibrate completely.

- Note: Heat block or water bath equilibration may require several hours, depending on device performance. **Failure to use proper temperature may result in decreased product performance.**

Mix the E-Sphere™ Stool Buffer by shaking before use. Mix the E-Sphere™ Stool Enzyme by inverting and centrifuge briefly before use.

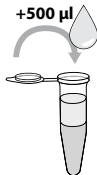
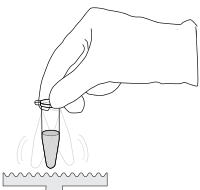

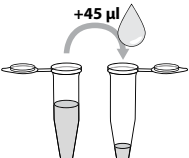
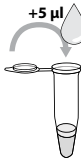
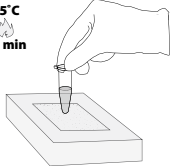
Obtain one E-Sphere™ Stool Clean-Up Column for every sample, mix by inverting and shaking. Remove the plug from each column, and place the column into a 1.5-ml microcentrifuge tube (see step 7 of Procedure). Allow columns to equilibrate at room temperature in an upright position during steps 1–6 of Procedure.


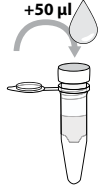
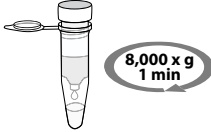
- Note: If performing >24 extractions, it is recommended to prepare columns before step 1. See step 7 of Procedure.

Measure 200 mg of fresh or frozen stool sample into a 1.5-ml microcentrifuge tube. For liquid stool samples, transfer 200 µl of sample into a 1.5-ml microcentrifuge tube.

The E-Sphere™ Stool DNA Extraction Kit is intended for use with fresh or frozen stool samples. The procedure is compatible with stool samples preserved with Cary Blair transport medium or low viscosity polyvinyl alcohol (LV-PVA). However, the use of preserved stool samples may decrease the sensitivity of downstream analysis. See the Troubleshooting section for more information on using preserved samples.

Procedure

1	<p>Add 500 µl of E-Sphere™ Stool Buffer to each sample.</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <ul style="list-style-type: none"> NOTE: To prevent contamination, use a new pipette tip for each sample. </div>	<p style="text-align: center;">+500 µl</p> 	Homogenize
2	<p>Vortex samples at full speed for 1–2 minutes or until fully homogenized.</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <ul style="list-style-type: none"> NOTE: For simultaneous extractions, the use of a multiple sample vortex attachment is recommended. </div>		
3	<p>Centrifuge samples at 200 x g for 5 seconds.</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <ul style="list-style-type: none"> NOTE: Please consult your centrifuge documentation for exact RCF to RPM conversions. </div>		Digest
4	<p>Transfer 45 µl of each sample supernatant into a new 1.5-ml microcentrifuge tube.</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <ul style="list-style-type: none"> NOTE: Discard unused sample according to your institutional biosafety guidelines. </div>	<p style="text-align: center;">+45 µl</p> 	
5	<p>Add 5 µl of E-Sphere™ Stool Enzyme to each sample. Pipette up and down several times to mix.</p>	<p style="text-align: center;">+5 µl</p> 	
6	<p>Incubate samples at 75 °C for 15 minutes in an equilibrated heat block, water bath, or thermal cycler.</p>	<p style="text-align: center;">75 °C 15 min</p> 	

7	<p>While samples are incubating, centrifuge the columns at 8,000 x g for 3 minutes. Transfer the prepared columns to new 1.5-ml microcentrifuge tubes. Discard the tubes containing flow-through.</p> <div data-bbox="222 315 612 488" style="border: 1px solid black; padding: 5px;"> <ul style="list-style-type: none"> ▪ NOTE: Column caps should not be removed or loosened prior to centrifugation. <p>The volume of flow-through should be ~450 μl. See Troubleshooting section for more information on preparing columns.</p> </div>	
8	<p>Centrifuge samples briefly after incubation. Transfer the entire volume of each sample (40–50 μl) directly into a prepared column and cap.</p> <div data-bbox="222 657 612 740" style="border: 1px solid black; padding: 5px;"> <ul style="list-style-type: none"> ▪ NOTE: Care should be taken to not disturb the contents of prepared columns. </div>	
9	<p>Centrifuge samples at 8,000 x g for 1 minute. Discard the used columns. The flow-through contains the purified DNA.</p> <div data-bbox="222 877 612 959" style="border: 1px solid black; padding: 5px;"> <ul style="list-style-type: none"> ▪ NOTE: Resulting DNA may be used immediately for PCR analysis or stored at $\leq -20^{\circ}\text{C}$ for later use. </div>	

Purify

Specifications

Starting material:	Up to 200 mg stool sample
Number of components:	3 components
Number of procedural steps:	9 steps
Number of sample transfers:	2 transfers
Total hands-on time for 12 extractions:	Less than 20 minutes
Total procedure time for 12 extractions:	Less than 30 minutes
Typical DNA yield (200 mg/ μ l stool):	50 – 200 ng/ μ l
Downstream compatibility:	PCR-based applications
Shelf life:	1 year at 4 °C

Troubleshooting

Issue	Solution
Insufficient sample supernatant in step 4	45 μ l sample supernatant is required in step 4. Some formed stool samples may not yield 45 μ l in this step. Add up to 1000 μ l of E-Sphere™ Stool Buffer vortex, and centrifuge per steps 2–3. This should yield adequate sample supernatant to proceed to step 5.
Additional DNA needed	The final volume of the purified DNA should be between 40-50 μ l. If additional volume is required for downstream analysis, multiple extractions may be performed from a single sample supernatant (step 4).
Poor downstream PCR performance	<ol style="list-style-type: none"><li data-bbox="513 649 886 839">1. Ensure that the heat block or water bath used for enzyme incubation have been properly equilibrated. Alternatively, a thermal cycler may be used to incubate samples during step 6.<li data-bbox="513 863 886 1053">2. Excessive centrifugation during step 3 may cause suspended microbial cells to pellet, reducing DNA yield. Take care to follow the centrifugation time and speed required by the procedure.
Black pellet/residue in purified DNA	Incorrectly prepared E-Sphere™ Stool Clean-Up Columns may allow small amounts of column material into purified DNA. In this case, transfer purified DNA into a new tube without disrupting the black pellet/residue. The presence of a black pellet/residue in the purified DNA (step 9) does not impact downstream PCR.

Issue	Solution
<p>Insufficient flow-through from columns (step 7)</p> <p>and/or</p> <p>Volume of purified DNA greater than 50 μl</p>	<p>Incorrectly prepared E-Sphere™ Stool Clean-Up Columns may have fluid retained in the column after the initial centrifugation step. In this case, columns should be re-centrifuged to ensure fluid is removed from column prior to adding digested samples.</p>
<p>Stool samples are preserved in Cary Blair transport medium or LV-PVA.</p>	<p>Preservatives must be removed using the following wash procedure prior to extraction:</p> <ol style="list-style-type: none"> 1. Centrifuge 200 mg (or 200 μl) samples at 10,000 x g for 2 minutes in round bottom 2-ml microcentrifuge tubes. 2. Remove the sample supernatants. 3. Add 500 μl of neutral phosphate buffered saline (PBS) to each sample. 4. Vortex samples until completely suspended. 5. Centrifuge samples at 10,000 x g for 2 minutes. 6. Remove the sample supernatants. 7. Repeat steps 3–6, for a total of two PBS washes. <p>After washing, the samples may be extracted according to the standard procedure.</p>



Material Safety Data Sheets

To obtain MSDS information, visit Phthisis Diagnostics online at www.phthisisdiagnostics.com or contact technical support at (434) 293-8180.

Warranty and Liability

Phthisis Diagnostics is committed to providing high quality products. Phthisis Diagnostics warrants that its products, if stored correctly and used properly, will meet or exceed the performance standards described under the Specifications section until the expiration date. Phthisis Diagnostics expressly disclaims all other warranties of any kind, express or implied. Phthisis Diagnostics' liability shall not exceed the purchase price of the product. Phthisis Diagnostics shall have no liability for indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products. See the full limited warranty statement that accompanies products for full terms, conditions and limitations of Phthisis Diagnostics limited product warranty, or contact Phthisis Diagnostics for a copy of this statement.

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Ordering Information

Phthisis Diagnostics products can be purchased at www.phthisisdiagnostics.com or by calling 434-293-8180.

Patents and Licensing Notifications

E-Sphere™ Stool DNA Extraction Kit is patent pending.

Trademarks

The Phthisis Diagnostics logo and E-Sphere™ are registered trademarks of Phthisis Diagnostics, Limited Liability Corporation. All other trademarks are the sole property of their respective owners.

Contact Information and Support

For information regarding Phthisis Diagnostics' products, to obtain technical support, to submit product suggestions, or for general inquiries please contact:

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