EZgene™ Stool gDNA Kit
Instruction Booklet (IFU)
(Cat. No. GD2417) v210711

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1. Introduction

The EZgene™ Stool gDNA Kit is designed for rapid and reliable purification of high-quality genomic DNA from various stool samples. Up to 1 gram of stool can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the ezBind™ matrix with a proprietary buffer system to eliminate PCR-inhibitory compounds such as humic acid from stool samples. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques.

This procedure avoids organic extraction, reducing plastic waste and hands-on time and enabling multiple samples to be processed in parallel.

In this procedure, the stool sample is homogenized and treated with a specially formulated detergent-containing buffer. Humic acid, proteins, polysaccharides, and other contaminants are precipitated after a heat/freeze step. DNA is further purified using a DNA spin column. Two rapid wash steps remove trace contaminants, and purified DNA is eluted in water or a low ionic strength Elution Buffer. Purified DNA can be directly used in downstream applications without further purification.

2. Storage and Stability

All components of the EZgene™ Stool gDNA Kit should be stored at 22°C–25°C. Under these conditions, DNA has been successfully purified and used for PCR after 12 months of kit storage.

During shipment or storage in cool ambient conditions, precipitates may form in some buffers. These deposits can be dissolved by incubating the solution at 65°C.

3. Kit Contents

Component	GD2417-00 (4 preps)	GD2417-01 (50 preps)	GD2417-02 (250 preps)
DNA Columns	4	50	250
Stool Vial	4	50	250
2 mL tubes	8	100	500
DH Reagent	1.2 mL	12 mL	60 mL
Buffer LX	5 mL	40 mL	200 mL
Buffer P2	1.5 mL	15 mL	60 mL
Buffer BL	3 mL	30 mL	150 mL
Elution Buffer	1.5 mL	20 mL	100 mL
DNA Wash Buffer	1 mL	15 mL	3 × 24 mL
RNase A	15 µL	160 µL	800 µL
Instruction Booklet	1	1	1

Safety Note:

Buffer BL contains chaotropic salts that may form hazardous compounds when mixed with bleach. Wear gloves and protective eyewear when handling this solution. Do not mix bleach with any kit solutions.

4. Before Starting

Please read the entire booklet before beginning the protocol.

- Preheat Buffer LX and Elution Buffer at 65°C. Ensure any crystals in Buffer LX are completely dissolved.
- Prepare DNA Wash Buffer by adding absolute ethanol as follows and store at room temperature:
    - Add 4 mL ethanol to GD2417-00 DNA Wash Buffer bottle
    - Add 60 mL ethanol to GD2417-01 DNA Wash Buffer bottle
    - Add 96 mL ethanol to GD2417-02 DNA Wash Buffer bottle

5. Stool DNA Isolation Protocol

Materials to be provided by the user

- Microcentrifuge capable of at least 12,000 × g
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water bath or heating block set to 70°C
- Absolute ethanol (96–100%)
- Isopropanol (100%)

6. Purification Protocol

1. Add 0.25–0.5 g stool sample to a Stool Vial. Vortex briefly (~5 seconds). Add 70 µL Buffer LX. Vortex at maximum speed for 5 minutes or until thoroughly homogenized.

2. Incubate at 70°C for 10 minutes. Mix twice during incubation by vortexing. Optional (Gram-positive bacteria): Incubate at 95°C for 2 minutes. Centrifuge at 10,000 × g for 1 minute. Transfer 500 µL of clear lysate to a 2 mL tube. Important: Make sure the Stool Vial rotates freely in the centrifuge without rubbing. Caution: Do not exceed 10,000 × g.

3. Add 250 µL Buffer P2, mix thoroughly by vortexing for 30 seconds, and incubate on ice for 5 minutes.

4. Centrifuge at 10,000 × g for 2 minutes. Carefully transfer ~600 µL supernatant (avoid the pellet) to a 1.5 mL tube.

5. Add 250 µL Buffer DH, mix well by vortexing for 5 seconds, and incubate on ice for 5 minutes.

6. Centrifuge at 10,000 × g for 2 minutes at room temperature.

7. Transfer ~700 µL supernatant (avoid pellet) to a clean tube. Add 1 mL Buffer BL and 100 µL isopropanol. Mix well by vortexing for 5 seconds.

8. Transfer 700 µL sample to a mini column and centrifuge at 10,000 × g for 30 seconds. Discard flow-through and reuse collection tube. Repeat to process remaining sample.

9. Add 700 µL DNA Wash Buffer to the column and centrifuge at 10,000 × g for 30 seconds. Discard flow-through and return column to the collection tube.

10. Centrifuge the empty column at maximum speed for 2 minutes to dry. Transfer the column to a clean 1.5 mL tube.

11. Add 50 µL Elution Buffer directly to the center of the membrane/matrix. Incubate at 65°C for 5 minutes.

12. Centrifuge at 10,000 × g for 1 minute to elute DNA.

13. Optional: Re-apply the eluate to the column and centrifuge again at 10,000 × g for 1 minute to increase yield.

7. Troubleshooting Guide

Problem	Possible Reason	Suggested Improvement
Low DNA yield or no DNA eluted	Sample stored incorrectly	Store samples at –20°C
	Poor homogenization	Repeat isolation with new sample. Mix thoroughly with Buffer LX and glass beads; increase bead-beating time to ensure complete lysis
	Incorrect Buffer BL addition	Repeat isolation with a new sample
	DNA washed off	Prepare DNA Wash Buffer correctly by adding ethanol (see “Before Starting”)
Problems in downstream applications	Ethanol residue in eluate	Ensure column is completely dried before elution
Little/no supernatant after initial centrifugation	Insufficient centrifugal force	Increase centrifugation time and/or verify centrifuge speed
Sample does not pass through column	Column clogging	Increase centrifugation time and verify centrifugal force
Low A260/230 ratio	Inhibitor carryover	Repeat isolation; mix thoroughly with DH reagent and perform DH extraction twice
	Salt contamination	Ensure column is dried; consider extra wash with DNA Wash Buffer
	Wash buffer ethanol % too low	Prepare wash buffer using 96–100% ethanol

8. Limited Use and Warranty

This product is warranted to perform as described in its labeling and in Biomiga literature when used in accordance with instructions. No other warranties of any kind, express or implied, including but not limited to implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga.

Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at Biomiga's option, to replace the product. Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, results of use, or inability to use the product.

For technical support or additional product information, please contact us at (858) 603-3219 or visit www.biomiga.com.