Technical Bulletin

GenElute™ Microbiome DNA Purification Kit

High yield for PCR, sequencing and NGS

MBD5000

Product Description

Study of the biological roles of gut bacteria requires accurate and reproducible microbial data.[1] The crucial step in this process is to apply an appropriate methodology to extract microbial DNA. Setting up the optimal DNA isolation procedure is critical for robustness and reproducibility of the results. Inaccurate DNA extraction may result in microbial misrepresentation.[2]

The GenElute™ Microbiome DNA Purification Kit provides a convenient and rapid method to isolate high molecular weight, high-quality and high yield microbial DNA from stool samples. The kit contains DNA-free reagents to assure that no external contamination is present.

The kit uses a combination of chemical, mechanical, and thermal lysis with a simple and rapid spin column procedure to purify the DNA. The purified DNA is high molecular weight and high-quality and is fully compatible with downstream applications such as PCR and NGS.

Typical DNA recovery is 5-25 µg from 0.2 g of human stool or 0.1 g of mouse stool.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Consult the Safety Data Sheet for information regarding hazardous and safe handling practices.

Storage/Stability

All solutions should be kept tightly sealed. The kit should be stored at room temperature. The unopened kit is stable for 2 years as supplied.

Components

This kit contains reagents sufficient for 50 DNA extractions.

Component	Component Number	Amount
M1 Lysis Buffer	MBD5001	40 mL
M2 Lysis Boost Buffer	MBD5002	10 mL
M3 Cleaning Buffer	MBD5003	15 mL
M4 Binding Buffer	MBD5004	45 mL
M5 Wash Buffer	MBD5005	26 mL
M6 Elution Buffer	MBD5006	10 mL
Columns	MBD5007	50
Collection Tubes	MBD5008	100
Lysis Beads Tubes	MBD5009	50
Short protocol card	MBD5010	1

Component Information

- Before first use, add 26 mL of 96-100% ethanol to the M5 Wash Buffer. Mix well.
- Prior to each use, inspect buffers M1 and M4 for precipitate. If precipitate is visible, warm the buffers at 37 °C for 10-15 minutes. Shake well to dissolve.

Equipment Required, But Not Provided

- Benchtop microcentrifuge
- Bead beater
- Vortex
- DNase free 1.5-2 mL microcentrifuge tubes
- 96-100% ethanol

Procedure

General notes

All centrifugation steps are carried out in a benchtop microcentrifuge. All centrifugation steps are performed at room temperature.

Sample preparation

- Best results are obtained when stool samples are freshly collected prior to DNA extraction.
- If this is not feasible, stool samples should be frozen and stored at –20 °C.
- If frozen immediately upon collection, samples are stable for at least 6 months.
- Samples should be homogenized prior to extraction.
- Recommended input amount:
  - Human stool: 0.2 ± 0.05 g
  - Mouse stool: 0.1 ± 0.05 g

Lysate preparation

1. Weigh the stool sample into the provided Lysis Beads Tube. Add an appropriate volume of **M1** Lysis Buffer:
   - Human stool: 0.6 mL
   - Mouse stool: 0.7 mL
2. Vortex thoroughly for at least 10 seconds to homogenize the sample.
3. Add 100 µL of **M2** Lysis Boost Buffer. Vortex.
4. Incubate at 65 °C for 10 minutes.
5. Set the bead beater to maximum speed and homogenize for 10 minutes.

   Note: In case a bead beater is not available, homogenization can be performed with a standard laboratory vortex. In such cases, set the vortex to maximum speed and homogenize for ≥ 20 min.
6. Centrifuge at 14,000 × *g* for 5 minutes.
7. Transfer 400 µL of the supernatant to a clean 1.5 mL microcentrifuge tube, being careful not to transfer the pelleted debris.
8. Add 250 µL of **M3** Cleaning Buffer. Vortex immediately.
9. Centrifuge at 14,000 × *g* for 2 minutes.
10. Transfer 500 µL of the supernatant to a clean 2 mL microcentrifuge tube, being careful not to transfer the pelleted debris.
11. Add 900 µL of **M4** Binding Buffer. Vortex.

Binding to Column

1. Assemble a spin column with a provided collection tube.
2. Load up to 700 µL of the sample mixture onto a spin column-tube assembly. Centrifuge at 14,000 × *g* for 1 minute.
3. Discard the flow-through. Reassemble the spin column with the collection tube.
4. Load the remaining sample mixture into the column. Centrifuge at 14,000 × *g* for 1 minute.
5. Ensure that the entire sample mixture has passed into the collection tube by inspecting the column. If sample is still present at the upper part of the column, centrifuge again at 14,000 × *g* for 1 minute.
6. Discard the flow-through and the collection tube.

Column Wash

1. Assemble the spin column with a clean collection tube. Add 500 µL of **M5** Wash Buffer.
2. Centrifuge the spin column-tube assembly at 14,000 × *g* for 1 minute.
3. Discard the flow-through. Reassemble the spin column with the collection tube.
4. Add 500 µL of **M5** Wash Buffer. Centrifuge the spin column-tube assembly at 14,000 × *g* for 1 minute.
5. Discard the flow-through. Reassemble the spin column with the collection tube.
6. Centrifuge the empty spin column-tube assembly at 14,000 × *g* for 0.5-1 minute.
7. Discard the flow-through and the collection tube.

DNA Elution

1. Assemble the spin column with a clean microfuge tube. Add 100 µL of **M6** Elution Buffer. Incubate at room temperature for 1 minute.

   Note: It is possible to elute the DNA with 200 µL of M6 Elution Buffer to increase the overall DNA yield. However, the DNA concentration will be reduced.
2. Centrifuge the spin column-tube assembly at 14,000 × *g* for 1 minute. Discard the column.

3. The purified DNA is in the tube and ready for use.
4. Store the purified DNA:
   - At 4 °C for up to 7 days.
   - At –20 °C for long-term storage.

References

1. Videnska, P. *et al., Sci. Rep.*, **9(1)**, 13837 (2019).
2. Fiedorová, K. *et al.*, *Front. Microbiol.* **10**, 821 (2019).

Troubleshooting Guide

Problem	Possible Cause	Recommendation
Low DNA yield	Too much sample input can result in low yield.	Ensure that the maximum input of stool is not exceeded
	Ineffective lysis.	Heat samples at 95 °C for 10 minutes.
	Incorrect sample handling	Minimize the time stool sample is outside the freezer before its processing
Inhibition of downstream applications	Presence of inhibitors in the eluted DNA.	Dilute the DNA before proceeding with downstream applications.
		Ensure that the maximum input of stool is not exceeded.
		Perform additional wash step
		Incubate on ice for 10 min after addition of M3 Cleaning Buffer.

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