Instruction Manual

SPINeasy DNA Kit for Tissue and Bacteria

(With Lysing Matrix)

Spin Column Kit for Easy Isolation of Genomic DNA from Animal Tissues, Blood, Cultured Cells and Bacteria in Less Than 20 Minutes

Cat. No.:

116532050 (50 Preps)

116532005 (5 Preps Sample Kit)

Storage: 15 – 30 °C

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1. Introduction

SPINeasy DNA Kit for Tissue and Bacteria is a high-performance genomic DNA (gDNA) extraction kit based on silica-membrane spin-column technology. This kit enables quick isolation of gDNA from various animal tissues, blood, cultured cells and bacteria, typically in less than 30 minutes. The use of our specially formulated Lysis Buffer GD and Lysing Matrix A in combination with FastPrep® Instruments from MP Biomedicals enables highly efficient lysis of various types of samples within seconds. Provided in the kit, Column GD and kit buffers are designed to deliver gDNA extracts of high yield and purity, compatible with downstream applications such as PCR, restriction digestion and sequencing. Visit www.mpbio.com to explore additional products to support your research.

2. Kit Components and User Supplied Materials

2.1 Kit Components

Components	Package	Cat. No.
Lysing Matrix A	50 ea	116910050
Lysis Buffer GD	60 mL	116532051
Wash Buffer GD1	30 mL	116532052
Wash Buffer GD2	6 mL	116532053
Elution Buffer GD	10 mL	116532054
Column GD with collection tubes	50 ea	116532056
Instruction Manual	1 ea	-
Quick-Start Protocol	1 ea	-
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500)
- Vortex (an adapter for multiple microcentrifuge-sized tubes is recommended if multiple samples are to be processed simultaneously), if FastPrep® Instrument is unavailable
- Microcentrifuge capable of at least 14,000 x g
- Heat block (optional)
- Absolute ethanol (50 mL)
- 1.5 mL Microcentrifuge tubes (1 per prep)
- Gram-positive bacteria pre-treatment buffer containing 20 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0, 1.2% Triton® X-100 and 20 mg/mL lysozyme. Store at 4 °C after adding lysozyme. Only required for DNA extraction from gram-positive bacteria.

3. Storage and Stability

All SPINeasy DNA Kit for Tissue and Bacteria components are guaranteed for at least 24 months from the date of manufacture when stored at room temperature (15 – 30 °C).

4. Notes Before Starting

Please check as appropriate:

- ☐ Add 50 mL (5 mL for sample kit) of absolute ethanol to Wash Buffer GD2 and mark on the bottle.
- ☐ Prepare one 1.5 mL microcentrifuge tube per prep for elution of purified genomic DNA.
- ☐ Lysis can be performed by vortexing the sample in a vial of Lysing Matrix A at the maximum speed if a FastPrep® Instrument is unavailable.
- ☐ Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 x g is not feasible.

5. Safety Precautions

Lysis Buffer GD contains a component that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink or smoke when using this product. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

A Quick-Start Protocol is provided in the kit for quick reference throughout the extraction process.

6.1 Pre-treatment of Gram-positive Bacteria

For DNA extraction from gram-positive bacteria, perform the following pre-treatment steps before proceeding to the DNA extraction protocol.

1. Prepare gram-positive bacteria pre-treatment buffer (to be supplied by user) containing:
    - 20 mM Tris-HCl, pH 8.0
    - 2 mM EDTA, pH 8.0
    - 1.2% Triton® X-100
    - 20 mg/mL lysozyme. Store at 4 °C after adding lysozyme.
2. Harvest cells by centrifugation at 10,000 x g for 3 mins, discard supernatant. Resuspend bacterial pellet in 200 µL of gram-positive bacteria pre-treatment buffer.
3. Incubate for 30 mins at 37°C.

6.2 DNA Extraction Protocol

1. Refer to Table 1 for recommended starting amounts and summary of sample preparation in Lysing Matrix A.
    - Animal tissues: Cut tissue into small pieces and transfer into a vial of **Lysing Matrix A**. Add 1 mL of **Lysis Buffer GD**.
    - Cultured cells and gram-negative bacteria cell pellet: Harvest cells by centrifugation at 10,000 x g for 3 mins, discard supernatant. Resuspend cell pellet in 1 mL of **Lysis Buffer GD** and transfer to a vial of **Lysing Matrix A**.
    - Blood and cell suspension: Add **Lysis Buffer GD** to make up to 1 mL final volume, mix and transfer the mixture into a vial of **Lysing Matrix A**.
    - Gram-positive bacteria: Follow the instructions in section 6.1 to perform pre-treatment. Add 800 µL of **Lysis Buffer GD**, mix and transfer to a vial of **Lysing Matrix A**.
2. Homogenize in a FastPrep® Instrument for 15 seconds at speed setting of 4.0 m/s.
   Alternatively, vortex samples at the maximum speed for 20 mins for animal tissues and bacteria or 10 mins for blood and cultured cells. It is recommended to perform vortexing with the use of an adapter to hold the vials, especially if multiple samples are to be processed simultaneously.
3. Centrifuge at 14,000 x g for 10 mins.
   Note: Centrifuge at the maximum speed for all steps if 14,000 x g is not feasible.
4. Transfer 750 µL of the supernatant to a **Column GD with collection tube**.
5. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
6. Add 500 µL of **Wash Buffer GD1** to the column.
7. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.

8. Add 750 µL of **Wash Buffer GD2** to the column. Incubate at room temperature for 1 min.
9. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
10. Centrifuge at 14,000 x g for an additional 1 min to dry column.
11. Optional: Incubate at 55°C for 3 – 5 mins to dry column completely.
12. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
13. Add 100 µL of **Elution Buffer GD** to the center of the membrane. Incubate at room temperature for 1 min. For samples with low DNA content, reducing the elution volume to 50 µL may increase the concentration of eluted DNA.
14. Centrifuge at 8,000 x g for 1 – 2 mins to elute DNA.
15. Eluted genomic DNA will be collected in the microcentrifuge tube.

Table 1: Summary of sample preparation in Lysing Matrix A.

Sample type	Starting amount*	Lysis Buffer GD	Procedure
Animal tissues	15 mg (up to 10 mg for spleen, 30 mg for other tissues)	1 mL	Cut tissue into small pieces, transfer to Lysing Matrix A and add 1 mL Lysis Buffer GD.
Blood (non-nucleated)	100 µL – 200 µL	Make up to 1 mL final volume	Add Lysis Buffer GD to make up to 1 mL final volume, mix and transfer the mixture into a vial of Lysing Matrix A.
Blood (nucleated)	10 µL	Make up to 1 mL final volume	Add Lysis Buffer GD to make up to 1 mL final volume, mix and transfer the mixture into a vial of Lysing Matrix A.
Cultured cell pellet	1 x 10^6 cells (up to 5 x 10^6 cells)	1 mL	Resuspend cell pellet in 1 mL of Lysis Buffer GD and transfer to a vial of Lysing Matrix A.
Cultured cells in 100 µL PBS	1 x 10^6 cells (up to 5 x 10^6 cells)	900 µL	Add Lysis Buffer GD to make up to 1 mL final volume, mix and transfer the mixture into a vial of Lysing Matrix A.
Gram-negative bacteria	Pellet from 3 mL to 9 mL of overnight culture	1 mL	Resuspend cell pellet in 1 mL of Lysis Buffer GD and transfer to a vial of Lysing Matrix A.
Gram-positive bacteria	Pellet from 3 mL to 9 mL of overnight culture	800 µL, following pre-treatment	Perform pre-treatment (Section 6.1), add 800 µL of Lysis Buffer GD, mix and transfer to a vial of Lysing Matrix A.

*The amount of starting material is provided as a guideline. Optimal amounts differ depending on the exact tissue type and are to be determined by the user.

7. Data

SPINeasy DNA Kit for Tissue and Bacteria has been rigorously tested for its performance. The following table displays gDNA yields obtained from various samples using the kit. Results demonstrate that gDNA extracted with this kit are suitable for PCR amplification.

Table 2: Quality and quantity of gDNA extracted from various sample types using SPINeasy DNA Kit for Tissue and Bacteria.

Sample	Starting Amount	Extraction Results		
		Yield (µg)	$A_{260/280}$	$A_{260/230}$
Heart	27 mg	16.60	1.85	2.79
Liver	15 mg	25.35	1.86	2.74
Spleen	8 mg	50.10	1.86	2.55
Lung	11 mg	17.17	1.84	2.54
Kidney	11 mg	22.23	1.85	2.86
Blood (Rabbit)	200 µL	2.48	1.84	2.16
Blood (Chicken)	10 µL	25.81	1.87	2.83
S. aureus	Pellet from 1.5 mL overnight culture	21.79	1.85	3.07
E. coli	Pellet from 6 mL overnight culture	6.92	1.80	1.47
HEK293T cells	1 x10^6 cells	12.15	1.86	2.14

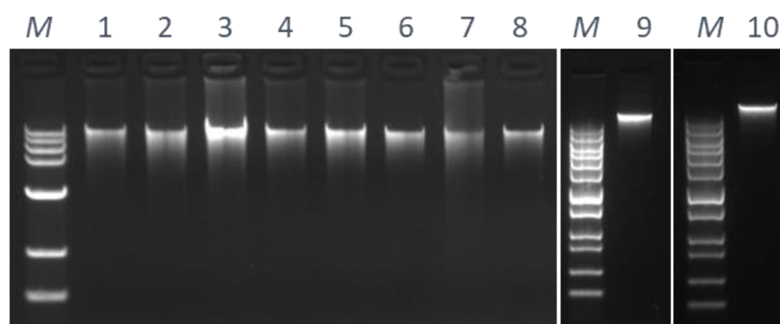

Figure 1 (left): gDNA extracted from various samples using SPINeasy DNA Kit for Tissue and Bacteria, analyzed using agarose gel electrophoresis. *M*: DNA marker; Lane 1: Heart; Lane 2: Liver; Lane 3: Spleen; Lane 4: Lung; Lane 5: Kidney; Lane 6: Rabbit blood; Lane 7: Chicken blood; Lane 8: *S. aureus*; Lane 9: *E. coli*; Lane 10: HEK293T cells.

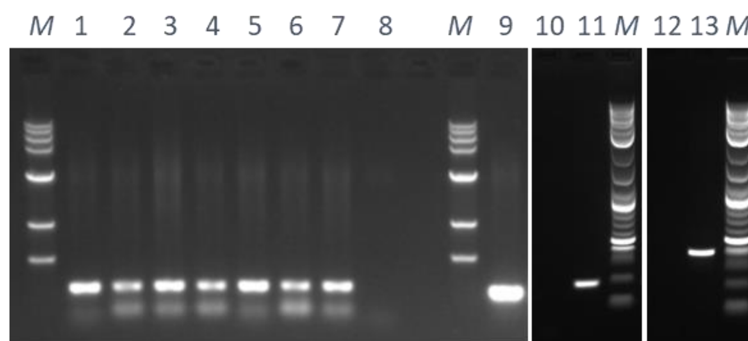

Figure 2 (right): PCR amplification of gDNA extracted from various samples using SPINeasy DNA Kit for Tissue and Bacteria. *M*: DNA marker; Lane 1: Heart; Lane 2: Liver; Lane 3: Spleen; Lane 4: Lung; Lane 5: Kidney; Lane 6: Rabbit blood; Lane 7: Chicken blood; Lane 8: Neg ctrl (β-actin); Lane 9: *S. aureus*; Lane 10: Neg ctrl (16S); Lane 11: *E. coli*; Lane 12: Neg ctrl (globin); Lane 13: HEK293T cells.

8. Troubleshooting

8.1 Low DNA Yields

1. Ensure the extraction was performed according to kit manual instructions.
2. Tissue with low DNA content: (i) Increase amount of starting material; (ii) Process multiple samples using several Lysing Matrix tubes and then pool the samples; (iii) Elute in a smaller volume (50 µL).
3. Insufficient lysis: While a FastPrep® speed setting of 4.0 m/s for 15 seconds is sufficient for most sample types, some samples may require harsher conditions for complete lysis. Homogenization speed and/or time can be increased for such samples. Lysis duration can also be extended when samples are lysed by vortexing.
4. Poor elution: Ensure that Elution Buffer GD is added to the center of the column membrane.
5. Ethanol carry-over: Incubate column at 55°C for 3 – 5 mins to dry the membrane completely before elution.

8.2 Low A_{260}/A_{280} Ratios for Purified DNA

1. Inaccurate readings due to low DNA concentration: Refer to 8.1.
2. Insufficient cell lysis: Refer to 8.1.3.
3. Contaminants not removed efficiently: After adding Wash Buffer GD2, incubate column at room temperature for 1 min before centrifuging.

8.3 High A_{260}/A_{280} Ratios for Purified DNA

1. Inaccurate readings due to low DNA concentration: Refer to 8.1.
2. RNA contamination: This kit is designed to purify DNA with high selectivity over RNA. However, if RNA contamination is evident and its removal is necessary, sample may be treated by adding RNase A to a final concentration of 100 µg/mL during sample lysis. RNase A is not provided in the kit.

8.4 Low A_{260}/A_{230} Ratios for Purified DNA

1. Inaccurate readings due to low DNA concentration: Refer to 8.1.
2. Contaminants not removed efficiently: After adding Wash Buffer GD2, incubate column at room temperature for 1 min before centrifuging.

8.5 Sheared DNA

1. Sample overlysis: Reduce FastPrep lysis speed and/or duration. Lysis using a vortex instead of a FastPrep will generally result in higher DNA integrity but compromised yields.
2. Sample degradation: Sample quality is critical to the integrity of purified DNA. For best results, DNA should be extracted from fresh samples or freshly frozen samples. It is recommended to store samples frozen in aliquots and avoid repeated freeze-thawing.

8.6 Coloured Eluate

Blood sample: Repeat Wash Buffer GD2 washing step if a coloured flow-through is observed.

9. Product Use Limitations & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s). FastDNA®, FastRNA®, FastPrep®, QBiogene®, and BIO 101® Systems are registered trademarks of MP Biomedicals, LLC.

10. Worldwide Ordering and Technical Support

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