Instruction Manual

SPINeasy DNA Kit for Water

Spin Column Kit for Quick Isolation of Genomic DNA from Water

Cat. No.: 116536050 (50 Preps)

Storage: 15-30 ˚C

Table of Contents

1. Introduction 3
2. Kit Components and User Supplied Materials 4
3. Storage and Stability 5
4. Notes Before Starting 5
5. Safety Precautions 5
6. Protocol 6
7. Data 7
8. Troubleshooting 8
9. Product Use Limitations & Warranty 9
10. Worldwide Ordering and Technical Support 10

1. Introduction

SPINeasy DNA Kit for Water is a high-performance water gDNA extraction kit based on silica-membrane spin-column technology. This kit enables quick isolation of gDNA from water in less than 30 min. Water samples are processed using our uniquely formulated Lysis Buffer W1 and Lysing Matrix E to effectively lyse various types of cells. Column W1 provided in the kit has high binding capacity and selectivity for gDNA. The combination of components in the kit extracts gDNA of high yield and purity that is ready for downstream analyses 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 E	50 ea	116994050
Lysis Buffer W1	60 mL	116536051
Lysis Buffer W2	8 mL	116536052
RNase A Solution	550 µL	116530053
Inhibitor Removal W	15 mL	116536053
Binding Buffer W	30 mL	116536054
Wash Buffer W1	9 mL	116536055
Wash Buffer W2	6 mL	116536056
DES Buffer	10 mL	116530057
Filter Membrane	50 ea	116536057
Column W1	50 ea	116536058
2.0 mL Collection Tubes	50 ea	116530059
1.5 mL Collection Tubes	50 ea	116530060
Instruction Manual	1 ea	-
Quick-Start Protocol	1 ea	-
MSDS & CoA	www.mpbio.com	

2.2 User Supplied Materials

- Vortex mixer with adapter
- Vacuum filter set
- Microcentrifuge capable of at least 14,000 x g
- Water bath or heat block
- Isopropanol (30 mL)
- Absolute ethanol (71 mL)
- 2.0 mL Microcentrifuge tubes (100 pcs)

3. Storage and Stability

All SPINeasy DNA Kit for Water 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:

- If Lysis Buffer W1 has precipitated, heat at 55 °C to dissolve precipitate.
- Add 30 mL isopropanol to Binding Buffer W and mark on the bottle.
- Add 21 mL absolute ethanol to Wash Buffer W1 and mark on the bottle.
- Add 50 mL absolute ethanol to Wash Buffer W2 and mark on the bottle.
- Filter Membrane for collection of microorganisms is provided in the kit.
- 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 W1 and W2 contain components that may cause irritation when in contact with human tissue. Binding Buffer W contains components that are corrosive and can cause severe skin burns. 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.

1. Filter water sample using vacuum filter set. Depending on the microbial load and turbidity of the water sample, try to obtain the highest amount of residue possible on the filter. Take note of the volume of sample used.
2. Using forceps, pick up the filter membrane and roll it into a cylinder shape with the top side (microbe trapping side) facing inwards, as shown in figure 1.

Figure 1: Preparation of filter membrane for DNA extraction

3. Insert the filter into a **Lysing Matrix E** tube.
4. Add 980 µL **Lysis Buffer W1**, 120 µL **Lysis Buffer W2**, 10 µL **RNase A** to the sample in the Lysing Matrix E tube and vortex at maximum speed for 10 min.
5. Add 250 µL **Inhibitor Removal W** to the Lysing Matrix E tube and mix by inverting the tube 20 times.
6. Centrifuge at 5,000 x g for 5 min to pellet precipitate. Transfer supernatant (up to 900 µL) to a clean 2 mL microcentrifuge tube (not provided).
7. Add an equal volume of **Binding Buffer W** to the supernatant in the 2 mL tube. Vortex to mix.
8. Transfer 800 µL of the mixture to **Column W1** placed on top of a **2.0 mL Collection Tube** (provided). Centrifuge at 14,000 x g for 30 s. Empty the collection tube. Repeat the process once.
9. Add 500 µL of **Wash Buffer W1** to Column W1. Centrifuge at 14,000 x g for 30 s. Empty the collection tube.
10. Add 500 µL of **Wash Buffer W2** to Column W1. Centrifuge at 14,000 x g for 30 s. Empty the collection tube. Repeat the wash process with Wash Buffer W2.
11. Without addition of any liquid, centrifuge at 14,000 x g for 2 min to dry the column.
12. Discard the collection tube and replace with a new, clean **1.5 mL Collection Tube**. Air dry the column for 5 min at room temperature.
13. Heat **DES Buffer** to 55 ˚C using a water bath while waiting.
14. Add 100 µL of pre-heated DES Buffer to center of the column.
15. Centrifuge at 14,000 x g for 1 min to bring eluted DNA into the clean collection tube. Discard the column. DNA is now ready for downstream applications. Store at -20 ˚C for extended periods or 4 ˚C until use.

7. Data

SPINeasy DNA Kit for Water has been thoroughly tested for its performance. The following table displays gDNA yields obtained from various water samples using the kit. Results demonstrate high yields of pure gDNA extracted and suitable for PCR amplification.

Table 1: Quality and quantity of gDNA extracted from various water samples using SPINeasy DNA Kit for Water.

Sample		Extraction Results		
	Filtrate Volume (mL)	Yield (ng/ µL)	$A_{260/280}$	$A_{260/230}$
River water	100	46.22	1.88	1.90
Pond water	165	19.85	1.86	2.32
Seawater	1000	28.39	1.92	2.00
Sewage	15	120.32	1.83	1.65

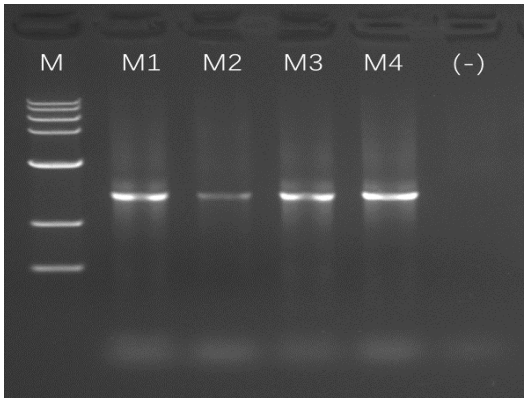

Figure 2: gDNA extracted from different types of water samples using SPINeasy DNA Kit for Water, analyzed using 1 % agarose gel electrophoresed at 70 V for 30 min. M: 1kb plus DNA ladder; Lane 1: River water (2 µL); Lane 2: Pond water (2 µL); Lane 3: Seawater (2 µL); Lane 4: Sewage (0.5µL).

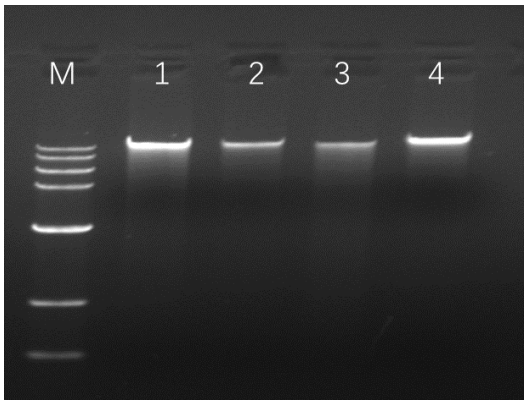

Figure 3: 16S- PCR amplification of gene from different types of water samples using SPINeasy DNA Kit for Water.
M: 1kb plus DNA ladder; Lane 1: River water; Lane 2: Pond water; Lane 3: Seawater; Lane 4: Sewage; Lane 5: Negative control.

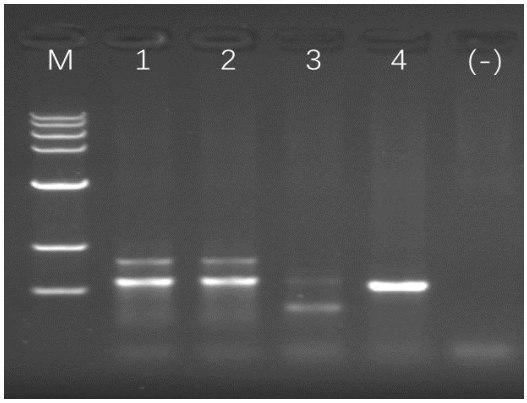

Figure 4: ITS-PCR amplification of gene from different types of water samples using SPINeasy DNA Kit for Water.
M: 1kb plus DNA ladder; Lane 1: River water; Lane 2: Pond water; Lane 3: Seawater; Lane 4: Sewage; Lane 5: Negative control.

8. Troubleshooting

8.1 Sample handling

1. Depending on the quantity of microbes in the water sample, carefully process the highest possible amount of sample until the filter is clogged and unable to filter further. Take note of the volume of sample used.
2. SPINeasy DNA Kit for Water is suitable for natural water, wastewater, lake water, pond water, glacier water, sewage, rainwater etc.

8.2 Low DNA Yields

1. Ensure the extraction was performed as per the manual protocol.
2. Low microbiological content: (i) Increase amount of starting material; (ii) If water sample is of high turbidity, employ an additional filtration step using filters with bigger pore sizes prior to filtering using the filter membrane in the kit. Filters with larger pore sizes can be stacked on top of the filter membrane. Employing filters with larger pore sizes will filter out large particles and allow the smaller pore size filter membrane to trap microorganisms. Filter the highest possible amount of sample through the filter membrane. This will allow for a higher amount of sample to be processed through the extraction kit; (iii) Increase vortex duration.
3. Increase DNA capture: Instead of transferring 2 x 800 µL of DNA-Binding Buffer W mixture to the column, transfer the entire volume.
4. Poor elution: (i) Ensure the DES Buffer is heated to 55 ˚C and is loaded to the center of the column during elution; (ii) Incubate the column with added DES Buffer for 5 min at 55 ˚C prior to elution.

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

1. Proteins not removed efficiently: Inhibitor Removal W must be efficiently mixed in the lysate. Invert tube by hand at least 20 times or mix by pipet pumping. Incubating the sample on ice/ keeping it in the fridge for 5 min can further precipitate proteins from difficult samples.
2. Contaminants not removed efficiently: Washing should be carried out twice using Wash Buffer W2.

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

Possible RNA contamination, which can be confirmed via gel electrophoresis analysis. Incubate sample with RNase A Solution for 5 min after the lysis step before spinning down the debris.

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

1. Proteins not removed efficiently: Refer to 8.3.1.
2. Contaminants not removed efficiently：Refer to 8.3.2.
3. Residual ethanol in eluted DNA: (i) Increase centrifugation speed or time to dry spin the column, (ii) Increase the air-drying time of Column S1 or (iii) Incubate the column in a 55 ˚C oven to speed up the drying process.

9. Product Use Limitations & Warranty

The products presented in this instruction manual are for research or further manufacturing use only. They are not to be used as drugs or medical devices 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

Australia
Tel: +61 2.8824.2100
Tel: +61 1800.249.998
Email: custserv.au@mpbio.com

Austria & Germany
Tel: 0800.426.67.337
Tel: 00800.7777.9999
Email: custserv.de@mpbio.com

Belgium
Tel: 00800.7777.9999
Email: custserv.be@mpbio.com

Canada
Tel: +1 800.854.0530
Email: custserv.ca@mpbio.com

China
Tel: +86 400.150.0680
Email: custserv.cn@mpbio.com

Europe
Tel: +33 3.88.67.54.25
Tel: +33 00800.7777.9999
Email: custserv.eur@mpbio.com

France
Tel: +33 3.88.67.54.25
Email: custserv.fr@mpbio.com

India
Tel: +91 22.27636921/22/25
Email: custserv.in@mpbio.com

Italy
Tel: 00800.7777.9999
Email: custserv.it@mpbio.com

Japan
Tel: +81 3.6667.0730
Email: custserv.jp@mpbio.com

Latin America
Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.la@mpbio.com

New Zealand
Tel: +64 9.912.2460
Email: custserv.nz@mpbio.com

North America
Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.na@mpbio.com

Poland
Tel: 00800.7777.9999
Email: custserv.po@mpbio.com

Russia
Tel: +7 495 604- 13- 44
Email: custserv.rs@mpbio.com

Serbia
Tel: +381 11.242.1972
Email: custserv.se@mpbio.com

Singapore/ APAC
Tel: +65 6775.0008
Tel: +65 6394.7675
Email: custserv.ap@mpbio.com

South Korea
Tel: +82 2.425.5991
Email: custserv.kr@mpbio.com

Switzerland
Tel: 00800.7777.9999
Email: custserv.ch@mpbio.com

The Netherlands
Tel: 00800.7777.9999
Email: custserv.nl@mpbio.com

United Kingdom
Tel: 0800.282.474
Email: custserv.uk@mpbio.com

www.mpbio.com