Instruction Manual
Research Use Only

SPINeasy DNA Kit for Water

Spin Column Purification for Easy Isolation of DNA from Water

Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116536050 (50 PREPS)
116536000 (5 PREPS)
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1. Introduction to SPINeasy DNA Kit for Water

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.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Vacuum manifold	Yes
Sample	Water
Sample amount	50-1000 mL (Depending on the quantity of microbes in the water sample)
Elution volume	100 µL

2. Kit Components and User Supplied Materials

2.1 SPINeasy DNA Kit for Water Component

	50 PREPS (Cat.No.: 116536050)		5 PREPS (Cat.No.: 116536000)	
Components	Package	Cat. No.	Package	Cat. No.
Equilibration Buffer	12 mL	116547059	1.2 mL	116547009
Lysing Matrix E	50 ea	116994050	5 ea	116994005
Lysis Buffer W1	60 mL	116536051	6 mL	116536001
Lysis Buffer W2	8 mL	116536052	0.8 mL	116536002
RNase A Solution	550 µL	116530053	55 µL	116570417
Inhibitor Removal W	15 mL	116536053	1.5 mL	116536003
Binding Buffer W	30 mL	116536054	3 mL	116536004
Wash Buffer W1	9 mL	116536055	0.9 mL	116536005
Wash Buffer W2	6 mL	116536056	0.6 mL	116536006
DES Buffer	10 mL	116530057	1 mL	116530007
Filter Membrane	50 ea	116536057	5 ea	116536007
Column W1	50 ea	116536058	5 ea	116536008
2.0 mL Collection Tubes	50 ea	116530059	5 ea	116530009
1.5 mL Collection Tubes	50 ea	116530060	5 ea	116530010
Quick-Start Protocol	1 ea	-	1 ea	-
Instruction Manual	Available www.mpbio.com			
MSDS & CoA	Available www.mpbio.com			

2.2 User Supplied Materials

- Microcentrifuge capable of at least 14,000 *g*
- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500) or Vortex.
- Metal MidiPrep™ adaptor (Cat. No.116002544)
- Vacuum filter set
- Water bath or heat block
- Isopropanol (30 mL)
- Absolute ethanol (71 mL)
- 2.0 mL microcentrifuge tubes (100 pcs)

- Single-channel pipettors (1 µL-1000 µL)
- Nuclease-free, aerosol-preventive tips
- Rack for microcentrifuge tube
- Biohazard disposal containers
- Personal Protective Equipment

3. Storage and Kit Stability

All the components and reagents of the SPINeasy Plasmid Miniprep Kit can be stored at room temperature (15-25˚C) until the expiration date printed on the kit label. For extended storage or storage in dry condition (humidity < 40%), store the columns at 2-8˚C to maintain performance.

4. Important Consideration Before Use

- ❑ If Lysis Buffer W1 has precipitated, heat at 55˚C to dissolve precipitate.
- ❑ Add 30 mL (3.0 mL for sample kit) of isopropanol to Binding Buffer W and mark the bottle.
- ❑ Add 21 mL (2.1 mL for sample kit) of absolute ethanol to Wash Buffer W1 and mark the bottle.
- ❑ Add 50 mL (5.0 mL for sample kit) of absolute ethanol to Wash Buffer W2 and mark 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 *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

1. **Column W1 Preparation**

   - Add **200 µL Equilibration Buffer** to the **Column W1** membranes to ensure its performance.

     Wait at least **1 min** and centrifuge for **10 sec @ maximum speed**. Transfer the Column SN into a new collection tube (provided).

2. **Sample processing**

   - 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.
   - 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. **Homogenize and Inhibitor Removal**

   - Insert the filter into a **Lysing Matrix E** tube.
   - Add **980 µL Lysis Buffer W1**, **120 µL Lysis Buffer W2**, and **10 µL RNase A** to the sample in the Lysing Matrix E tube. Homogenize using a Fastprep® at 6 m/s for 30s for 2 cycles, pause for 2 minutes between cycles with a Metal MidiPrep™ 5 mL metal adaptor (Cat. No. 116002544) or vortex for **10 min @ maximum speed.**
   - Add **250 µL Inhibitor Removal W** to the Lysing Matrix E tube and mix by inverting the tube 20 times.
   - Centrifuge for **5 min @ 5,000 *g*** to pellet the precipitate. Transfer supernatant (up to **900 µL**) to a clean 2 mL microcentrifuge tube (not provided).

4. **Bind**

   - Add an equal volume of **Binding Buffer W** to the supernatant in the 2 mL tube.

Vortex to mix.

- Transfer **800 µL** of the mixture to **Column W1** placed on top of a **2.0 mL Collection Tube** (provided). Centrifuge for **30 s @ 14,000 *g***. Empty the collection tube. Repeat the process once.

5. **Wash**
   - Add **500 µL Wash Buffer W1** to Column W1. Centrifuge for **30 s @ 14,000 x *g***. Empty the collection tube.
   - Add **500 µL Wash Buffer W2** to Column W1. Centrifuge for **30 s @ 14,000 x *g***. Empty the collection tube. Repeat the wash process with **Wash Buffer W2**.
   - Without addition of any liquid, centrifuge for **2 min @ 14,000 *g*** to dry the column.
   - Discard the collection tube and replace it with a new, clean **1.5 mL Collection Tube**. Air dry the column for **5 min @ room temperature**.
6. **Elution**
   - Heat DES Buffer to **55˚C** using a water bath while waiting.
   - Add **100 µL pre-heated DES Buffer** to center of the column.
   - Centrifuge for **1 min @ 14,000 *g*** 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.
   -

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

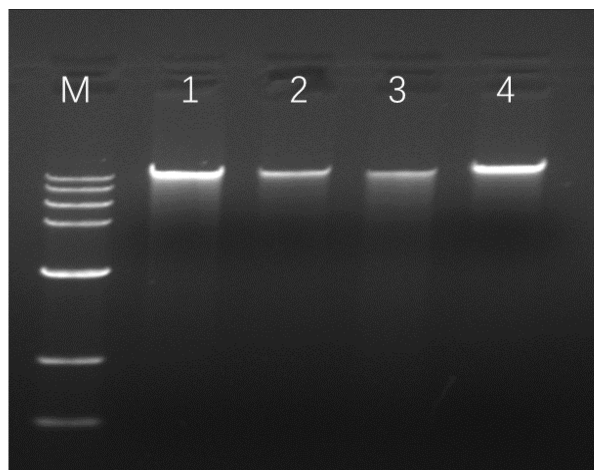

Figure 2: **gDNA extracted from different types of water samples using SPINeasy DNA Kit for Water, analyzed using 1 % agarose gel electrophoresed at 70 V for 30 min.** M: 1kb plus DNA ladder; Lane 1: River water (2 µL); Lane 2: Pond water (2 µL); Lane 3: Seawater (2 µL); Lane 4: Sewage (0.5µL).

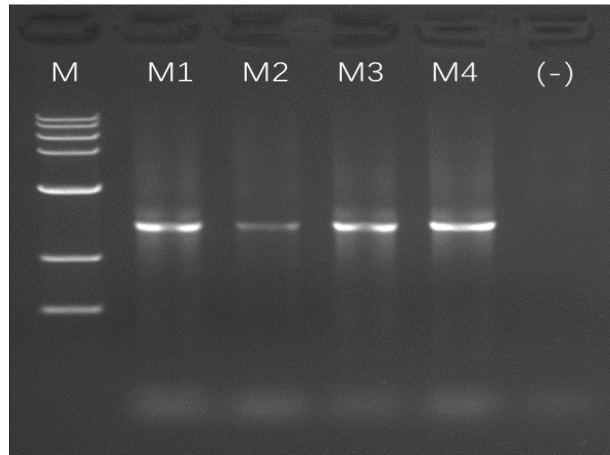

Figure 3: **16S- PCR amplification of gene from different types of water samples using SPINeasy DNA Kit for Water.** M: 1kb plus DNA ladder; Lane 1: River water; Lane 2: Pond water; Lane 3: Seawater; Lane 4: Sewage; Lane 5: Negative control.

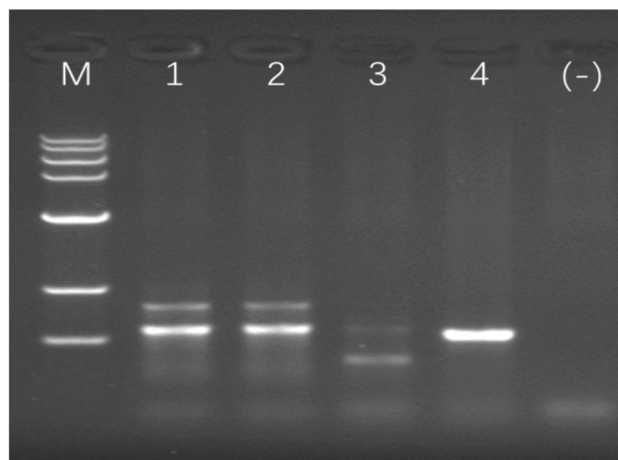

Figure 4: **ITS-PCR amplification of gene from different types of water samples using SPINeasy DNA Kit for Water.** M: 1kb plus DNA ladder; Lane 1: River water; Lane 2: Pond water; Lane 3: Seawater; Lane 4: Sewage; Lane 5: Negative control.

8. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at **apac-techsupport@mpbio.com**

Problem	Possible Cause	Recommendation
Sample handling	Various quantity of microbes in the water sample	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. SPINeasy DNA Kit for Water is suitable for natural water, wastewater, lake water, pond water, glacier water, sewage, rainwater etc.
Low DNA Yield	Inefficient extraction	Ensure the extraction was performed as per the manual's protocol.
	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.
	Insufficient DNA capture	Instead of transferring 2 x 800 µL of DNA-Binding Buffer W mixture to the column, transfer the entire volume.

	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.
Low A_{260}/A_{280} ratio	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.
	Contaminants not removed efficiently	Washing should be carried out twice using Wash Buffer W2.
High A_{260}/A_{280} ratio	Possible RNA contamination	Confirm RNA contamination via gel electrophoresis analysis. Incubate sample with RNase A Solution for 5 min after the lysis step before spinning down the debris.
Low A_{260}/A_{230} ratios	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.
	Contaminants not removed efficiently	Washing should be carried out twice using Wash Buffer W2.
	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 incubate the column in a 55 ˚C oven to speed up the drying process.

9. Product Use Limitation & 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 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.

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