Instruction Manual
Research Use Only

SPINeasy Plasmid Midiprep Kit

Spin Column Purification for Easy Isolation of Plasmid from Bacteria

Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116539025 (25 PREPS)
116539000 (2 PREPS)
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1. Introduction to SPINeasy Plasmid Midiprep Kit

SPINeasy Plasmid Midiprep Kit is a silica-membrane spin-column kit for the purification of high-copy and low-copy number plasmid DNA from 25 – 50 mL of transformed bacterial culture. Using our specially formulated buffers, bacterial cells are disrupted by alkaline lysis to release plasmid DNA. Cell debris, such as chromosomal DNA and proteins, are precipitated by neutralization and cleared through centrifugation. Subsequently, plasmid DNA in the lysate supernatant is adsorbed on a silica membrane, washed and eluted in a convenient process using the spin column technology. Up to 1 mg of plasmid DNA can be purified through this simple workflow, which is immediately ready for routine molecular biology laboratory applications.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Midi spin column
Sample	Bacterial cell pellet
Elution volume	500 µL

2. Kit Components and User Supplied Materials

2.1 SPINeasy Plasmid Midiprep Kit Component

Components	25 PREPS (Cat.No.: 116539025) Package	Cat. No.	2 PREPS (Cat.No.: 116539000) Package	Cat. No.
Equilibration Buffer	12 mL	116547059	1.2 mL	116547009
Resuspension Buffer SD1	60 mL	116539021	5 mL	116539001
Alkaline Lysis Buffer SD2	60 mL	116539022	5 mL	116539002
Neutralization Buffer SD3	90 mL	116539023	8 mL	116539003
Wash Buffer DW1	70 mL	116539024	7 mL	116539004
Wash Buffer DW2	12 mL	116539026	1.2 mL	116539006
Elution Buffer SD	30 mL	116539027	3 mL	116539007
RNase A	600 µL	116539028	50 µL	116539008
Column SD with collection tubes	25 ea	116539029	2 ea	116539009
Quick-start protocol	1 ea	-	1 ea	-
Instruction Manual	Available www.mpbio.com			
MSDS & CoA	Available www.mpbio.com			

2.2 User Supplied Materials

- Centrifuge with rotor and adaptors for 15 mL and 50 mL centrifuge tubes
- Isopropanol (30 mL)
- Absolute ethanol (100 mL)
- 15 mL centrifuge tubes (2 per prep)
- 1.5 mL microcentrifuge tubes (1 per prep)
- 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 Midiprep 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

- ❑ Briefly spin down the vial of RNase A and add the entire solution to Resuspension Buffer SD1. Mark the bottle and store Resuspension Buffer SD1 with RNase A in 4˚C.
- ❑ Add 30 mL (3 mL for sample kit) of isopropanol to Wash Buffer DW1 and mark the bottle.
- ❑ Add 100 mL (10 mL for sample kit) of absolute ethanol to Wash Buffer DW2 and mark the bottle.
- ❑ Prepare two labelled 15 mL microcentrifuge tubes per prep: one for lysate binding preparation and another for elution of plasmid DNA.
- ❑ Prepare one labelled 1.5 mL microcentrifuge tube per prep for storage of eluted plasmid DNA.

5. Safety Precautions

Neutralization Buffer SD3 and Wash Buffer DW1 contain substances 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

1. Growth of Bacterial Culture

- Pick a single transformed bacterial colony or scrape out a small amount of frozen bacterial glycerol stock and inoculate into 1 – 5 mL of LB medium containing the appropriate selective antibiotic. Incubate for 5 – 8 hours at 37 °C with shaking at 180 – 250 rpm.
- Dilute the starter culture 1/500 to 1/1000 in fresh LB medium containing the appropriate selective antibiotic and incubate overnight (approximately 16 hours) at 37 °C with shaking at 180 – 250 rpm. A culture volume of 25 – 50 mL per prep is recommended. Use a flask or vessel at least 4 times the volume of the culture.
- The recommended bacterial culture optical density (OD) for plasmid DNA isolation is around 2 – 3 (OD600 = 2 – 3). For more accurate determination of OD, dilute a small amount of the culture 5 times to obtain an OD600 reading that falls within 0.3 – 0.8, then multiply the value by the dilution factor to derive the actual OD of the undiluted culture.
- Harvest the cells by centrifugation for **20 mins @ 3,000 *g***. Discard the supernatant.
- The bacterial pellet can be used immediately or stored at -20 °C.

2. Column SD Preparation

- Add **450 µL Equilibration Buffer** to the **Column SD** membranes to ensure its performance.

  Wait at least **1 min** and centrifuge for **30 sec @ 14,000 *g***. Discard flow through and reuse collection tube.

3. Lysis and Neutralization

- Resuspend bacterial cell pellet in **2 mL Resuspension Buffer SD1**.
- Add **2 mL Alkaline Lysis Buffer SD2** and mix well by inverting the tube several times.

  Note: *Mixing should be performed gently; avoid vortexing or pipetting vigorously.*
- Proceed to the next step directly or within **5 min**. Do not exceed **5 min** of incubation time.

- Add **3 mL Neutralization Buffer SD3** and mix well by inverting several times.

  Note: *Mixing should be performed gently; avoid vortexing or pipetting vigorously.*
- Centrifuge for **10 min @ 15,000 *g***.

  Note: *Centrifuge at the maximum speed if 15,000* ***g*** *is not feasible. At lower speeds, longer centrifugation times may be necessary to pellet down cell debris.*
- Transfer the lysate supernatant into a new **15 mL centrifuge tube**. Avoid picking up the debris.

  Note: *If debris is excessive and unavoidable, spin down the supernatant an additional time to separate the remaining debris or filter the lysate (filter not provided).*

4. **Bind**
   - Add **2 mL ethanol** to the lysate supernatant and mix by inverting the tube.
   - Load **3.5 mL** mixture onto a Column SD with collection tube.
   - Centrifuge for **2 min @ 5,000 *g*** or until all the fluid has passed through the column. Discard flow through and reuse collection tube.
   - Repeat the loading and centrifugation steps until all lysate has been loaded.

5. **First Wash**
   - Add **3.5 mL Wash Buffer DW1** to the column.
   - Centrifuge for **2 min @ 5,000 *g*** or until all the fluid has passed through the column. Discard flow through and reuse collection tube.

6. **Second Wash.**
   - Add **3.5 mL Wash Buffer DW2** to the column.
   - Centrifuge for **2 min @ 5,000 *g*** or until all the fluid has passed through the column. Discard flow through and reuse collection tube.
   - Centrifuge for **an additional 10 min @ 5,000 *g*** to dry column.
   - Air dry membrane by allowing the column to stand for **3 min @ room temperature**.

7. **Elution**
   - Transfer Column SD into a clean 1.5 mL centrifuge tube. Add **500 µL Elution Buffer SD** to the middle of Column SD. Incubate for **1 min @ room temperature** and centrifuge for **5 min @ 5,000 *g*** to elute DNA.

     Eluted plasmid DNA will be collected in the centrifuge tube. Transfer the eluted plasmid DNA into a clean 1.5 mL microcentrifuge tube for easier storage.

Optional: *To obtain higher yield, perform a second elution with* ***500 µL Elution Buffer SD****.*

7. Data

The following are examples of plasmid extraction results using SPINeasy Plasmid Midiprep Kit. Plasmids of high yield and purity are obtained and are suitable for downstream molecular biology applications.

Table 1: **Yield and quality of plasmids purified using SPINeasy Plasmid Midiprep Kit.**

Vector Backbone	Plasmid Size (bp)	Culture Volume (mL)	Extraction Results		
			Yield (µg)	$A_{260/280}$	$A_{260/230}$
pWPI	11,103	25	196.52	1.87	2.24
pcDNA6	5,149	25	229.27	1.92	2.28
pcDNA3.1	7,459	25	640.06	1.91	2.33
pcDNA3.1	7,459	50	1088.56	1.91	2.34

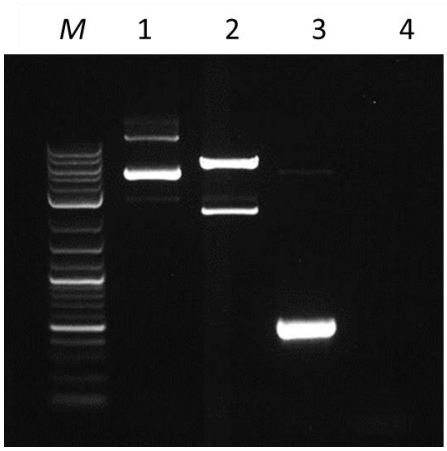

Figure 1: **Agarose gel electrophoresis result of pcDNA3.1 plasmid DNA extracted using the SPINeasy Plasmid Midiprep Kit with downstream molecular applications.** M: DNA marker; Lane 1: Purified plasmid DNA; Lane 2: Plasmid digested with two single cut restriction enzymes, yielding two bands; Lane 3: PCR of plasmid, producing a band of the expected amplicon size; Lane 4: PCR 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
Low DNA Yield	Inefficient extraction	Ensure the extraction was performed according to kit manual instructions. Ensure that the correct volumes of isopropanol and ethanol have been added to Wash Buffer DW1 and Wash Buffer DW2, respectively.
	Insufficient lysis	(i) Do not exceed 50 mL culture volume; (ii) Resuspend cell pellet completely in Resuspension Buffer SD1 before adding Alkaline Lysis Buffer SD2.
	Poor elution	Ensure that Elution Buffer SD is added to the centre of the column membrane.
	Ethanol carry-over	Incubate column at 55 °C for 3 – 5 mins to dry the membrane completely before elution.
	Bacterial stock is too old	Use freshly transformed bacterial colony to inoculate starter culture.
High A_{260}/A_{280} ratio	Inaccurate readings due to low DNA concentration	Ensure that the extraction was performed according to the kit manual's instructions.
	RNA contamination	Ensure that RNase A has been added to the Resuspension Buffer SD1.
Column clogging	Fluid does not pass through column completely	Extend centrifugation time until fluid passes through completely. Centrifugation speed may be increased if necessary.
	Large culture volume	Reduce culture volume. Larger culture volumes have higher tendencies to clog the column.

Genomic DNA contamination	Genomic DNA is sheared.	Do not vortex or pipette vigorously when performing alkaline lysis and neutralization (Step 3). Mix the solution by gently inverting the tube 5 – 8 times.
	Cells are over-lysed.	Do not exceed 5 minutes of alkaline lysis time (step 3).
	Cells are overgrown.	Do not cultivate cells for longer than 16 hours.
RNA Contamination	RNA contamination.	Ensure that RNase A has been added to Resuspension Buffer SD1. Store Resuspension Buffer SD1 at 4°C after adding RNase A.

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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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

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