Instruction Manual
Research Use Only

MagBeads Plasmid Miniprep Kit

(Ready-to-Use for MPure-96™)

Magnetic Beads-based Purification of Plasmid

Storage: 15-25 °C
Cat. No.: 117040200 (96 Preps)
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1. Introduction

The **Ready-to-Use MagBeads Plasmid Miniprep Kit** offers a scalable and automatable purification method using magnetic bead technology, suitable for extracting both high and low copy number plasmids. This kit efficiently yields plasmid DNA (2–20 µg) from 0.5–5 mL of overnight *E. coli* cultures, leveraging paramagnetic beads for rapid processing. Its magnetic technology and Ready-to-use format enhance user-friendliness, making it ideal for high-throughput automated nucleic acid purification.

The kit includes components for cell lysis and neutralization. Bacterial cells are first resuspended in Resuspension Buffer. Following resuspension, the cells are lysed with Alkaline Lysis Buffer to release plasmid DNA into the solution. Contaminants such as proteins and chromosomal DNA are precipitated by the Neutralization Buffer. The supernatant, which contains the plasmid DNA, binds to the magnetic particles, and a subsequent wash with an alcohol-containing buffer effectively removes impurities, resulting in purified plasmid DNA.

This kit is compatible with the MPure-32™ aNAP System, or other compatible high throughput instruments, facilitating a faster extraction process that takes approximately 40–50 minutes.

Visit www.mpbio.com to explore additional products to support your research.

Kit Specifications at a Glance

Technology	Magnetic Beads Automation Technology
Format	Magnetic Beads
Sample	Bacteria (Plasmid)
Sample amount	0.5 – 5 mL of bacterial culture
Observed yield	< 20 µg
Preparation time	< 50 minutes

2. Kit Components and User Supplied Materials

2.1 Ready-to-use MagBeads Plasmid Miniprep kit Components

Product	Package (Cat. No. 117040200)	Catalog No
Resuspension Buffer MN1*	26 mL	117040101
Alkaline Lysis Buffer MN2	26 mL	117040102
Neutralization Buffer MN3	37 mL	117040103
Binding Buffer MN	96 Preps	117040201
Wash Buffer MN	96 Preps	117040202
Elution Buffer MN	96 Preps	117040203
Elution Buffer MN	8 mL	116587056
96 Spin Tips	1 ea	117034111
RNase A	260 µL	117040105
Indicator Solution	130 µL	117040106
Quick-Start Protocol	1 each	-
Instruction Manual	Available www.mpbio.com	
MSDS & CoA	Available www.mpbio.com	

* For preparation and storage conditions see section 3

2.2 User Supplied Materials

- Standard microbiological equipment for growing and harvesting bacteria (Culture tubes, temperature controlled shaking incubator)
- Bacteria growth media and appropriate antibiotics
- Microcentrifuge capable of centrifugation of 1.5 mL / 2 mL tubes at a speed up to 16,000 g
- 1.5 mL microcentrifuge tubes
- Micropipette and micro tips
- Nuclease free microcentrifuge tubes
- 96–100 % Ethanol
- Isopropyl alcohol
- Personal protective equipment

3. Storage and Kit Stability

The MagBeads Plasmid Miniprep Kit is guaranteed until the expiry date stated on the kit when stored at room temperature (15-25˚C). Store the Elution Buffer MN plate at 2-8˚C upon receive. Do not freeze it. **Store Resuspension Buffer MN1** at 2-8˚C **after adding RNase A.**

4. Important Consideration Before Use

- ❑ Add **RNase A** and **Indicator Solution** (provided in the kit) to Resuspension Buffer MN1. Mark on the bottle after addition and store at 2-8˚C.
- ❑ Sodium dodecyl sulfate (SDS) in **Alkaline Lysis Buffer MN2** can precipitate at temperatures below 20˚C. In that scenario, incubate the buffer at 37˚C and mix.

5. Safety Precaution

Neutralization Buffer MN3, **Binding Buffer MN** and **Wash Buffer MN** contain chaotropic salts, which can form highly reactive compounds when combined with bleach. They can react with bleach and result in the generation of harmful / toxic cyanide or chlorine gases. These buffers also contain hazardous components 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. Liquid waste must be considered infectious and discarded according to the local safety guidelines.

Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

This protocol is for the automated purification of plasmid DNA from a bacterial culture volume of 0.5–5 mL. For high-copy-number plasmid, a culture volume of ≤2 mL is recommended, while for low-copy-number plasmid, a culture volume of ≤5 mL is recommended.

Things to do before starting

- All vortexing and centrifugation steps are carried out at room temperature (15-25˚C).
- Warm **Alkaline Lysis Buffer MN2** at 37˚C water bath if precipitate is noted.
- Add **RNase A** and **Indicator Solution** (provided with the kit) to **Resuspension Buffer MN1**.

Procedure:

Growth and pretreatment of bacterial cultures:

1. Inoculate **5 mL** of medium with bacteria (from agar plate) containing plasmid with appropriate antibiotic. Incubate at **37º C for 12-16 hr** overnight with shaking at **200-250 rpm**.
2. Ensure the bacterial culture reaches an OD_{600} of 2–3. Centrifuge **0.5-5 mL** of the overnight cultured bacterial sample at **13,000 rpm for 3 min**. Discard supernatant.
3. To the cell pellet, add **250 µL of Resuspension Buffer MN1** (containing 100 µg/mL of RNAse A) to resuspend the pellet. Resuspend the cells by pipetting.
4. Add **250 µL of Lysis Buffer MN2** and mix well by gently inverting 5-10 times or until the solution becomes clear. The solution will become viscous, so careful in handling to avoid sample contamination.

   Note: The solution will become viscous; handle carefully to avoid contamination. Do not vortex or pipette vigorously. If Indicator Solution is included in Resuspension Buffer MN1, the mixture will turn purple upon adding Alkaline Lysis Buffer MN2. Lysis time must not exceed 5 minutes.
5. Add **350 µL of cold (4 ˚C) Neutralization Buffer MN3** and mix immedately by gently inverting 5-10 times.

   Note: Note: Mix gently and thoroughly after addition of Neutralization Buffer MN3. Avoid vortexing or pipetting vigorously. If Indicator Solution is added to the Resuspension Buffer MN1, the lysate will turn from purple to yellow without any pink traces upon complete neutralization. This step will precipitate all protein and

chromosomal DNA completely.

6. Centrifuge at **13,000 rpm for 10 min** and carefully collect the clear lysate supernatant (approximately ~ 800 µL).

Plasmid extraction:

This protocol is for automated purification of plasmid DNA from total bacterial culture volume of 0.5-5 mL. For high-copy-number plasmids, a culture volume of ≤2 mL is recommended, while for low-copy-number plasmids, a culture volume of ≤5 mL is recommended.

MPure-96™ aNAP System Procedure:

1. Prepare clear lysate supernatant as described in section 6 (**Growth and pretreatment of bacterial cultures)** and add **lysate** to Binding Buffer MN**.**
2. Other reagents are provided as outlined in the table below:

Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 6	Plate 8
Sample / Buffer	Supernatant Binding Buffer MN	Wash Buffer MN	Wash Buffer MN	Wash Buffer MN	Magnetic Beads R Elution Buffer MN	96 Spin Tips
Buffer volume (µL)	~ 800 200	800	800	800	20 120	0

Note: Plate number corresponds to the plate locations in MPure-96™ instrument.

3. In MPure-96™ aNAP System, select the program “Plasmid” and adjust buffer volume as indicated above.
5. Otherwise, you may set the following program on other nucleic acid extraction instrument using the below parameters: For other nucleic acid extraction instruments, configure the program using the parameters listed below:Enter the protocol and parameters to start the run.

Step	Plate	Process	Time (s)			Mixing Speed	Temp (°C)	Pause
			Mix	Wait	Attract			
1	#6	Magnetic Beads wash	30	0	120	Medium	RT	OFF
2	#1	Bind	300	0	120	Medium	RT	OFF
3	#2	Wash	150	0	90	Medium	RT	OFF
4	#3	Wash	150	0	90	Medium	RT	OFF
5	#4	Wash	150	0	120	Medium	RT	OFF
6	#4	Dry	0	300	0	-	RT	OFF
7	#6	Elute	300	0	180	Medium	70	OFF
8	#2	Magnetic Beads abandon	0	0	0	Medium	RT	OFF

6. After completing the run, collect the eluates in a clean 1.5 mL micro centrifuge tube and store the tubes at -20°C for long term storage.
7. (Optional) Centrifuge eluted plasmid at **13,000 rpm** for **1 min** to remove magnetic beads residue.

7. Flow Chart

Automated Extraction using MPure-96™ aNAP System

Sample preparation

Inoculate bacteria colonies into culture tubes and grow culture at 37°C @ 220 rpm shaking for 16-24 hrs

3 min @ 13,000 rpm to pellet the bacteria

Lysis

Resuspend pellet with **250 µL Resuspension Buffer MN1**

Add **250 µL Alkaline Lysis Buffer MN2**. Mix by gently inverting 5-10 times or until the solution becomes clear (time should not exceed 5 min)

Add **350 µL col (4°C) Neutralization Buffer MN3**. Mix immediately by gently inverting 5-10 times.

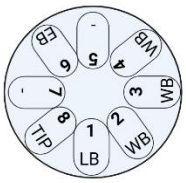
10 min @ 13,000 rpm

Collect clear lysate supernatant

Extraction

For Automated Extraction using MPure-96™ aNAP System:

① Transfer clear lysate supernatant into Binding Buffer MN plate



② Place all prefilled 96-well plates according to the assigned number

③ Select program "PLASMID" on MPure-96™ aNAP system

Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 6	Plate 8
Sample / Buffer	Supernatant / Binding Buffer MN	Wash Buffer MN	Wash Buffer MN	Wash Buffer MN	Magnetic Beads R / Elution Buffer MN	96 Spin Tips
Buffer volume (µL)	~ 800 / 200	800	800	800	20 / 120	0

④ Choose program "PLASMID" and run the extraction.

MPure-96™ aNAP System (PLASMID)

Step	Plate	Process	Time (s)			Mixing Speed	Temp (°C)	Pause
			Mix	Wait	Attract			
1	#6	Magnetic Beads wash	30	0	120	Medium	RT	OFF
2	#1	Bind	300	0	120	Medium	RT	OFF
3	#2	Wash	150	0	90	Medium	RT	OFF
4	#3	Wash	150	0	90	Medium	RT	OFF
5	#4	Wash	150	0	120	Medium	RT	OFF
6	#4	Dry	0	300	0	-	RT	OFF
7	#6	Elute	300	0	180	Medium	70	OFF
8	#2	Magnetic Beads abandon	0	0	0	Medium	RT	OFF

8. Data

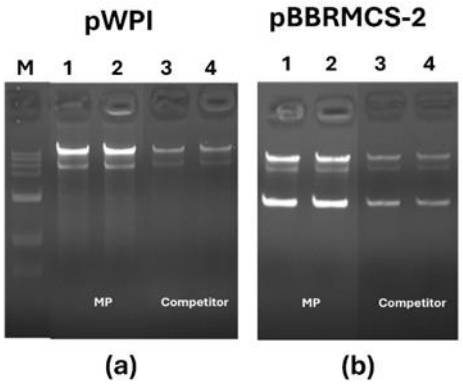


Figure 1. Agarose gel electrophoresis comparing plasmid DNA extracted using the MagBeads Plasmid Miniprep Kit (Ready-to-Use for MPure-96™) and Competitor O kit. (a) Results for pWPI plasmid. (b) Results for pBBRMCS-2 plasmid. Lane M: DNA marker. Lane 1-2: Plasmid DNA extracted with the MagBeads Plasmid Miniprep Kit **(Ready-to-Use for MPure-96™)**. Lane 3-4: Plasmid DNA extracted manually with the Competitor O kit.

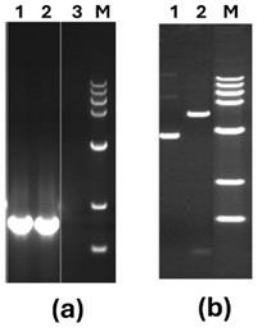


Figure 2. Agarose gel electrophoresis of plasmids extracted using the MagBeads Plasmid Miniprep Kit (Ready-to-Use for MPure-96™), demonstrating downstream applications: (a) PCR amplification and (b) restriction digestion with *EcoRI* and *NotI*.

(a)
Lane 1-2: PCR products using manually extracted plasmid DNA as template.
Lane 3: Negative control (no template).
Lane M: DNA marker.

(b)
Lane 1: Uncut plasmid.
Lane 2: Plasmid digested with *EcoRI* and *NotI*.
Lane M: DNA marker.

9. Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA Yield	Insufficient lysis	Resuspend cell pellet completely in Resuspension Buffer MN1 before adding Alkaline Lysis Buffer MN2.
	Precipitation in Alkaline Lysis Buffer MN2 before use.	Warm at 30 - 40°C for 5 min to dissolve precipitate and mix well before use.
	Insufficient neutralization	Mix thoroughly after adding Neutralization Buffer MN3. The solution will turn yellow if neutalization is complete. No pink coloration observed.
	Loss of DNA during extraction	Ensure magnetic Beads retrieved completely after each step.
	Prolonged exposure to Neutralization Buffer MN3	Affect the solubility of plasmid DNA. If plasmid DNA is not adequately separated from precipitated contaminants, it may be lost during subsequent washing steps
	Elution conditions	If nuclear free water is used, check pH.
	Growth medium other than LB may increase/decrease bacterial cell density.	Take appropriate amount of cell pellet.
	Bacteria stock is too old	Use freshly transformed bacterial colony to inoculate culture.
	Cells are overgrown	Do not cultivate cells for >OD_{600} of 3. Do not culture cells for more than 16 hr at 37°C in shaker. If rich medium is used, do not culture for more than 12 h.
	Plasmid has low copy number	Increase culture volume for plasmid purification.
No plasmid DNA	Storage of plasmid	Quantitate plasmid DNA concentration immediately after extraction or store at < 4°C if DNA is eluted in Tris buffer or at <-20°C if dissolved in water.
	Improper addition of sample to 96 Deep well plates	Ensure supernatant is added to the designated wells.
	Improper parameter settings in Automation instruments	Ensure correct parameter settings are set before running the procedure
	Reagent plates are not set in the designated place	Ensure plates are placed in the right orientation (columns A – H)
	Inaccurate readings due to low DNA yield	Refer to above

Low $A_{260/280}$ and $A_{260/230}$ ratios	Contaminants not removed efficiently	Ethanol not dried up completely in dry step.
		Ensure the tube caps are clean to prevent carryover of buffers from previous steps. Spin down or pipette out the contents from the microcentrifuge tube when placed in the magnetic stand to avoid contaminants.
	Excess bacterial culture volume used	Do not exceed 5 mL culture volume.
High $A_{260/280}$	RNA contamination	Ensure that RNase A has been added to Resuspension Buffer MN1 and store at 2-8°C after addition of RNase A.
Poor plasmid quality	Genomic DNA is sheared	Do not vortex or pipette vigorously during addition of Lysis Buffer MN2 and Neutralization Buffer MN3. When mixed too vigorously or for too long, genomic DNA may co-precipitate with plasmid DNA. Lysis step should not exceed 5 min. Mix solution by gently inverting plate/tube 5-8 times.
	Denatured plasmid DNA	Lysis and neutralization step should not exceed 5 min. Prolonged exposure will irreversibly denature plasmid DNA and interfere in downstream applications like PCR, Restriction digestion, sequencing etc.
	Cells are over lysed	Do not exceed 5 min of alkaline lysis time.
	Prolonged storage of cultures	Use freshly transformed bacterial colony to inoculate culture. Or harvest cells freshly after 16 hr and store the pellet at <-20°C until use.
Very high $A_{260/280}$	Magnetic Beads not removed during elution	Increase Magnetic bead retrieval step during elution
		Centrifuge at 15,000 g for 1 min to remove magnetic beads residue.
Show good amount of plasmid in nanodrop but not relatable to agarose gel	Plasmid degradation and genomic DNA contamination	Lysis and neutralization step should not exceed 5 min. Prolonged exposure will irreversibly denature plasmid DNA and interfere in downstream applications like PCR, Restriction digestion, sequencing etc.
	RNA contamination	Ensure that RNase A has been added to Resuspension Buffer MN1 and store at 2-8°C after addition of RNase A.

10. 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 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.

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