

MagBeads Plasmid Miniprep Kit

Magnetic Beads-based Purification of Plasmid

Size: 50 Preps / 5 Preps
Storage: 15-25 °C
Cat. No.: 116587050 (50 Preps) /
116587000 (5 Preps)
Content Version: Dec 2024

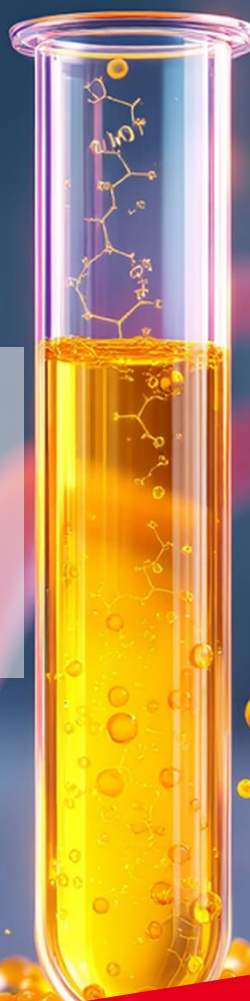


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1. Introduction

The 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 enhances user-friendliness, making it ideal for both small sample sizes and 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™ or MPure-96™ 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 MagBeads Plasmid Miniprep kit Components

Product	50 Preps (Cat. No. 116587050)		5 Preps (Cat. No. 116587000)	
	Package	Catalog No.	Package	Catalog No.
Resuspension Buffer MN1*	14 mL	116587051	1.4 mL	116587001
Alkaline Lysis Buffer MN2	14 mL	116587052	1.4 mL	116587002
Neutralization Buffer MN3	19 mL	116587053	1.9 mL	116587003
Magnetic Beads R*	1.1 mL	116583057	110 µL	116583007
Binding Buffer MN**	9 mL	116587054	0.9 mL	116587004
Wash Buffer MN**	2×8.5 mL	116587055	1.8 mL	116587005
Elution Buffer MN	8 mL	116587056	0.8 mL	116587006
RNase A	140 µL	116587057	14 µL	116587007
Indicator Solution	70 µL	116587058	7 µL	116587008
Quick-Start Protocol	1 each	-	1 each	-
Instruction Manual	Available www.mpbio.com			
MSDS & CoA	Available www.mpbio.com			

* For preparation and storage conditions see section 3

**For preparation see section 3

2.2 User Supplied Materials

For manual extraction

- Standard microbiology 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.
- MP Biomedicals™ Magnetic rack 8 or 24.
- Vortex or orbital shaker.
- 1.5 mL microcentrifuge tubes.
- Heating - block.
- Micropipette and micro tips.

- Nuclease free microcentrifuge tubes.
- 96 - 100 % Ethanol.
- Isopropyl alcohol.
- Personal protective equipment.

For automated extraction

- 6-tube format Autotube for MPure-32.
- 96 deep-well plate for MPure- 32 / 96.
- Cover for magnetic rod.

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 Magnetic Beads R and at 2-8°C upon receive. Do not freeze the Magnetic Beads R. **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. Label the bottle after addition and store at 4°C.
- Add 4.5 mL (50 Preps) / 450 µL (5 Preps) of isopropanol to Binding Buffer MN. Label the bottle after addition.
- Add 70 mL (50 Preps) of absolute ethanol to each bottle of Wash Buffer MN. For 5 preps, add 15 mL of absolute ethanol. Label the bottle after addition.
- 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.
- To ensure high yield and purity, perform a quick spin to collect droplets from tube (or collect using pipette and mix) after each vortex/mixing step.

5. Safety Precaution

Neutralization Buffer MN3 and Binding 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

Manual protocol

This protocol is for the manual 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 in the kit) to **Resuspension Buffer MN1**.
- Add indicated amount of isopropanol (100%) to **Binding Buffer MN** and absolute ethanol (95-100%) to **Wash Buffer MN**.

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 **37°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 μ L** of **Resuspension Buffer MN1** (containing RNase A) to resuspend the pellet. Resuspend the cells by pipetting.
4. Add **250 μ L** of **Alkaline Lysis Buffer MN2** and mix well by gently inverting 5-10 times 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 immediately by gently inverting 5-10 times.

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:

1. Transfer clear lysate supernatant to a clean microcentrifuge tube, add **200 µL of Binding Buffer MN** and mix 4-5 times by inversion.
2. Add **20 µL of Magnetic Beads R** to the supernatant containing Binding Buffer MN.
3. Mix by vortexing for **5 min at 1,500 rpm**.
4. Keep the tubes on magnetic rack for at least **1 min** and carefully discard the cleared supernatant using pipette.

IMPORTANT: Avoid disturbing the captured beads while aspirating the supernatant. To achieve higher washing efficiency, decant the remaining supernatant completely using a micro pipette

5. Add **800 µL of Wash Buffer MN** and mix by vortexing at **2,500 rpm for 3 min**.
6. Keep the tubes on magnetic rack for **1 min** and carefully discard the supernatant using a pipette.

IMPORTANT: Avoid disturbing the captured beads while aspirating the supernatant. To achieve higher washing efficiency, decant the remaining supernatant completely using a micro pipette.

7. Repeat steps 5-6 twice.
8. Keep the heat block set at **70° C** for elution step.
9. Air dry the magnetic beads for **5 min at room temperature**.

IMPORTANT: It is critical to completely remove all traces of buffer but be cautious not to over dry the beads as this will reduce the yield. Remove the remaining buffers as much as possible using a 10 µL micropipette.

Note: Residual ethanol may interfere with downstream applications. Ensure ethanol is completely removed during any subsequent washing steps to avoid contamination.

10. Add 120 μ L of Elution Buffer MN, mix and incubate at 70° C for 5 min at 1,000 rpm.
11. Keep the tubes on magnetic rack for 2 min or until the beads are retracted completely (the eluate should be clear).
12. Collect the eluates in a clean 1.5 mL micro centrifuge tube and store the tubes at -20°C for long term storage.
13. (Optional) Centrifuge eluted plasmid at 13,000 rpm for 1 min to remove magnetic beads residue.

Automated protocol

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-32™ aNAP System Procedure:

1. Prepare clear lysate supernatant as described in section 6 (Growth and pretreatment of bacterial cultures) and add lysate to wells #2/#8 in a 6-tube format autotube or a 96 deep-well plate.
2. Add the reagents into the respective well as outlined in the table below:

Well #	Well 1/7	Well 2/8	Well 3/9	Well 4/10	Well 5/11	Well 6/12
Sample / Buffer	-	Supernatant	Wash Buffer MN	Wash Buffer MN	Wash Buffer MN	Magnetic Beads R
		Binding Buffer MN				Elution Buffer MN
Buffer volume (μ L)	-	~ 800	800	800	800	20
		200				120

3. For MPure-32™ aNAP System: Select the program “Plasmid” and adjust the buffer volume as indicated above.
4. For other nucleic acid extraction instruments, configure the program using the parameters listed below:

Step	Well	Process	Time (s)			Mixing Speed	Temp (°C)	Pause
			Mix	Wait	Attract			
1	#6/#12	Magnetic Beads wash	30	0	120	Medium	RT	OFF
2	#2/#8	Bind	300	0	120	Medium	RT	OFF
3	#3/#9	Wash	150	0	90	Medium	RT	OFF
4	#4/#10	Wash	150	0	90	Medium	RT	OFF
5	#5/#11	Wash	150	0	120	Medium	RT	OFF
6	#5/#11	Dry	0	300	0	-	RT	OFF
7	#6/#12	Elute	300	0	180	Medium	70	OFF
8	#2/#8	Magnetic Beads abandon	0	0	0	Medium	RT	OFF

5. Enter the protocol and parameters to start the run.
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.

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 **96 deep-well plate # 1**. Add the reagents into the respective plates as outlined in the table below :

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

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

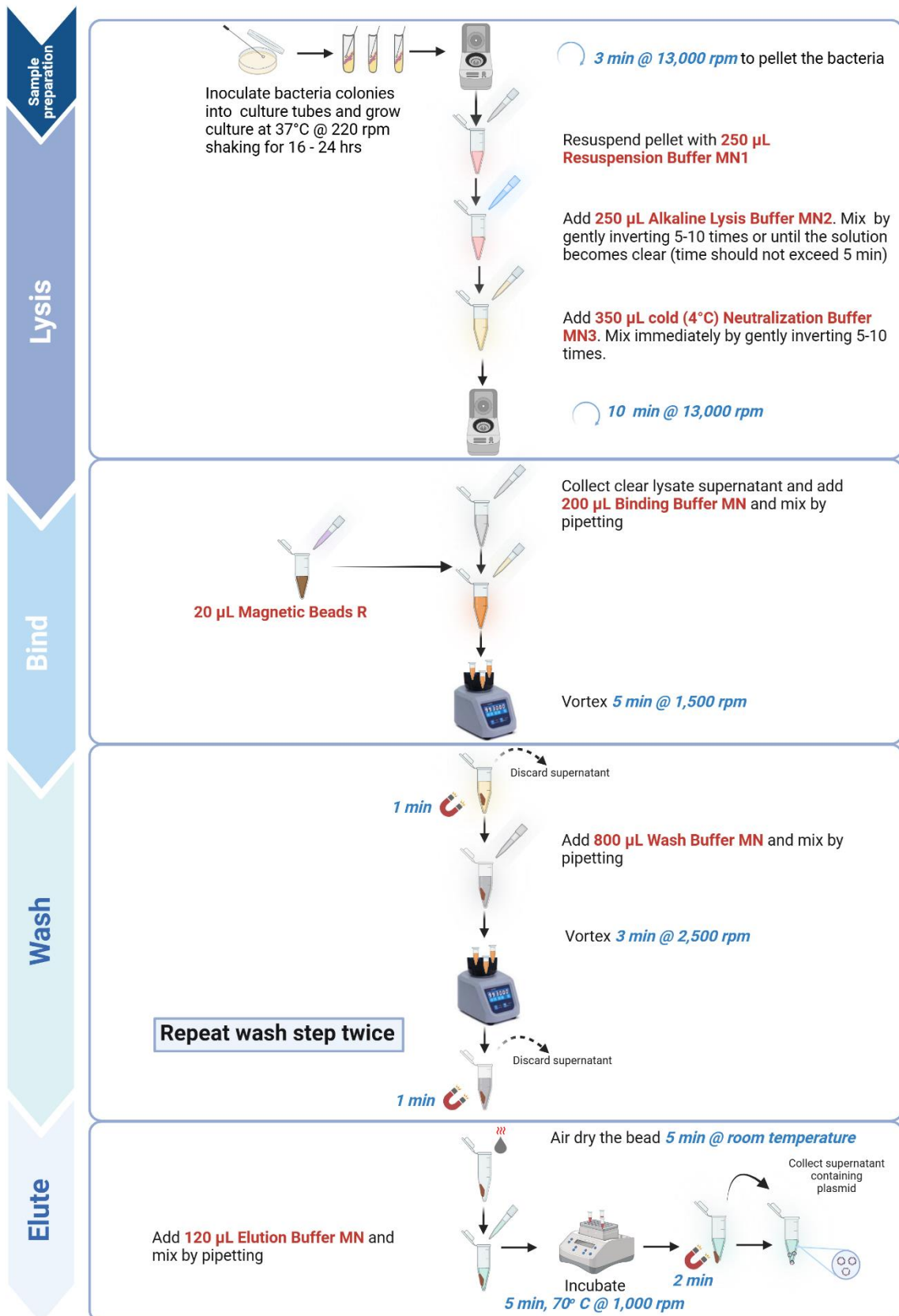
2. For MPure-96™ aNAP System, choose “Plasmid” and adjust buffer volume as indicated above. For other nucleic acid extraction instruments, configure the program using the parameters listed below:
3. Enter the protocol and parameters to start the run.

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

4. 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.
5. (Optional) Centrifuge eluted plasmid at 13,000 rpm for 1 min to remove magnetic beads residue.

7. Flow Chart

Manual Extraction

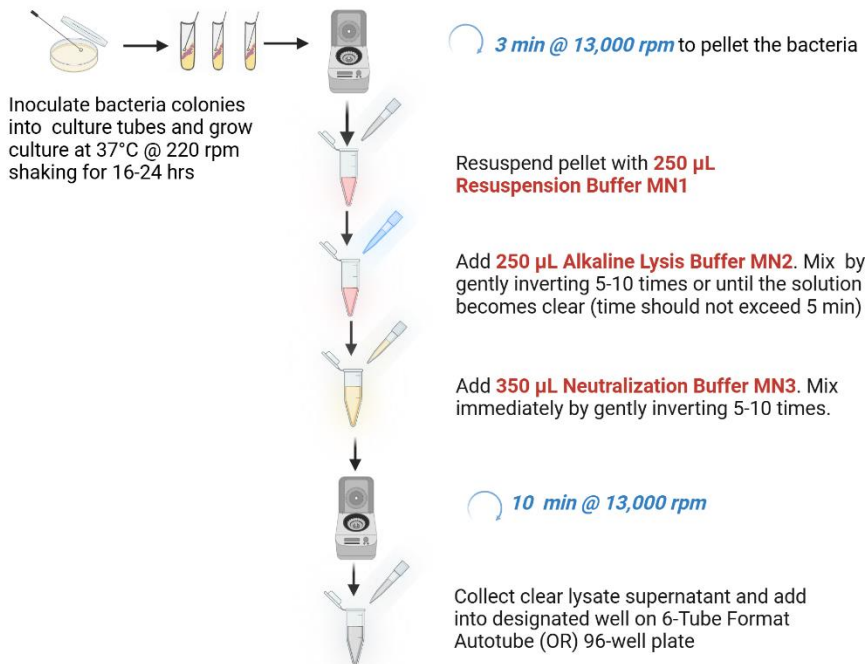


Automated Extraction using MPure-32™ aNAP System

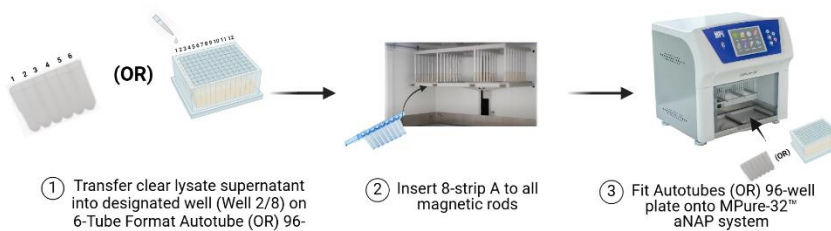
Sample preparation

Lysis

Extraction



For Automated Extraction using MPure-32™ aNAP System:



Well #	Well 1/7	Well 2/8	Well 3/9	Well 4/10	Well 5/11	Well 6/12
Sample / Buffer	-	Supernatant Binding Buffer MN	Wash Buffer MN	Wash Buffer MN	Wash Buffer MN	Magnetic Beads R Elution Buffer MN
Buffer volume (µL)	-	~ 800 200	800	800	800	20 120

④ Select program "PLASMID" and run the extraction.



MPure-32™ aNAP System (PLASMID)

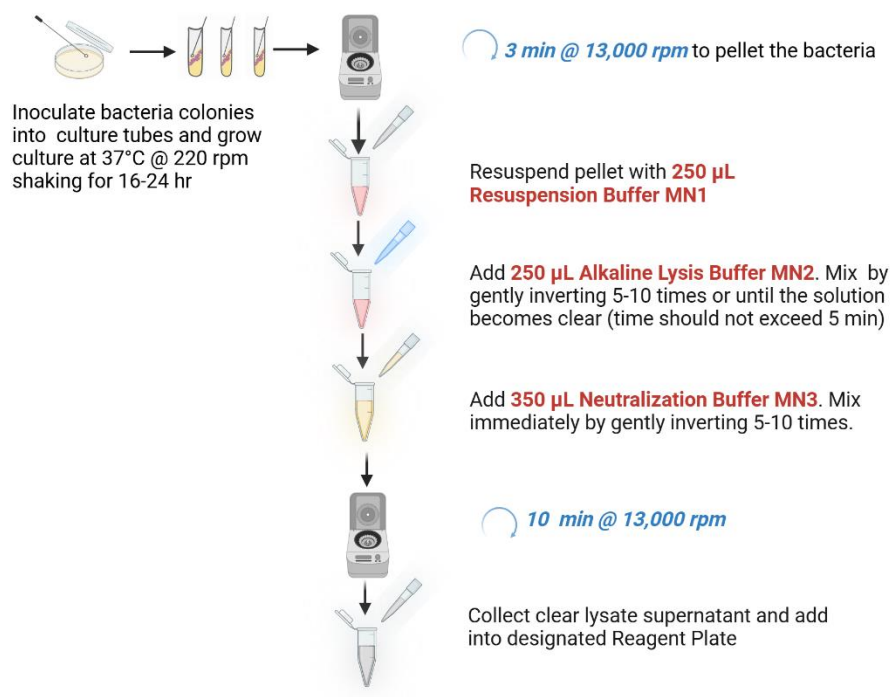
Step	Well	Process	Time (s)			Mixing Speed	Temp (°C)	Pause
			Mix	Wait	Attract			
1	#6/#12	Magnetic Beads wash	30	0	120	Medium	RT	OFF
2	#2/#8	Bind	300	0	120	Medium	RT	OFF
3	#3/#9	Wash	150	0	90	Medium	RT	OFF
4	#4/#10	Wash	150	0	90	Medium	RT	OFF
5	#5/#11	Wash	150	0	120	Medium	RT	OFF
6	#5/#11	Dry	0	300	0	-	RT	OFF
7	#6/#12	Elute	300	0	180	Medium	70	OFF
8	#2/#8	Magnetic Beads abandon	0	0	0	Medium	RT	OFF

Automated Extraction using MPure-96™ aNAP System

Sample preparation

Lysis

Extraction



For Automated Extraction using MPure-96™ aNAP System:

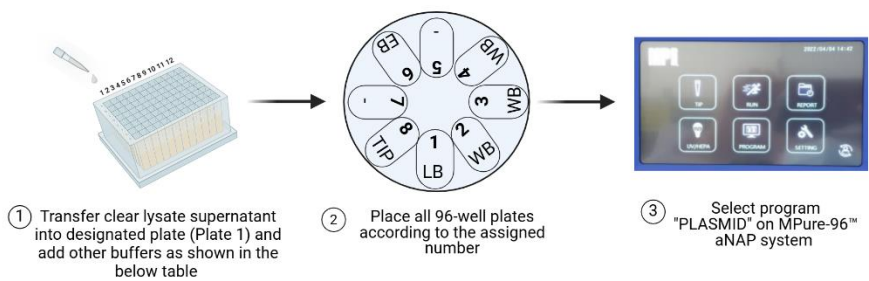


Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 6	Plate 8
Sample / Buffer	Supernatant Binding Buffer MN	Wash Buffer MN	Wash Buffer MN	Wash Buffer MN	Magnetic Beads R Elution Buffer MN	96 Spin Tips
Buffer volume (µL)	~ 800 200	800	800	800	20 120	0

④ Select 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

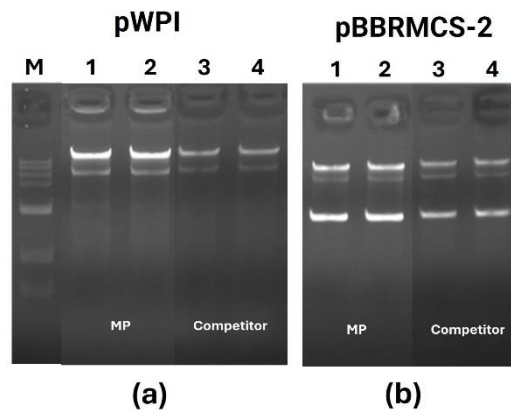


Figure 1. Agarose gel electrophoresis comparing plasmid DNA extracted using the MagBeads Plasmid Miniprep Kit and Competitor O kit. (a) Results for pWPI plasmid. (b) Results for pBBRMCS-2 plasmid. Lane M: DNA marker. Lane 1-2: Plasmid DNA extracted manually with the MagBeads Plasmid Miniprep Kit. Lane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

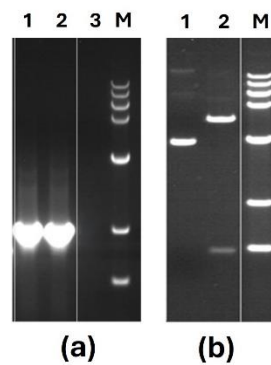


Figure 2. Agarose gel electrophoresis of plasmids extracted using the MagBeads Plasmid Miniprep Kit, demonstrating downstream applications: (a) PCR amplification and (b) restriction digestion with *EcoRI* and *NotI*.

(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 37 °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 neutralization is complete. No pink coloration observed.
	Loss of DNA during extraction	Ensure Magnetic Beads R is retrieved completely before discarding supernatant 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	Check pH of the elution buffer is 8. 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.
No plasmid DNA	Plasmid has low copy number	Increase culture volume for plasmid purification.
	Improper buffer preparation	Add recommended volume of isopropanol or ethanol to Binding Buffer MN and Wash Buffer MN respectively and mix well.
	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 buffers to autotubes or 96 deep well plates	Ensure correct buffers are 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 correct buffers are added to the designated wells.
Low $A_{260/280}$ and $A_{260/230}$ ratios	Inaccurate readings due to low DNA yield	Refer to above.
	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 Resuspension Buffer MN1 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 Alkaline 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 P1 Resuspension Buffer and store P1 Resuspension Buffer 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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