

MagListo™ **5M Plasmid Extraction Kit**



Cat. No. K-3600
K-3601



***MagListo*[™] 5M Plasmid Extraction Kit**

Kit for the extraction of plasmid DNA from bacterial culture

User Guide

K-3600	K-3601
 500	 100

Version No.: 4 (2022-05-25)

Please read all the information in booklet before using the unit



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Intended Use

MagListo™ 5M Plasmid Extraction Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

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All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Patent

MagListo™ and its kits are protected by the patents KR10-2015-0089172.

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Product Information

Components

Components	K-3601* (100 reactions)	K-3600** (500 reactions)	Storage
RNase A powder, lyophilized	3 mg x 1 ea	12 mg x 1ea	Store at room temperature (15-25°C).
Magnetic Nano Bead	9 ml x 1 ea	40 ml x 1 ea	
PM1 Buffer (Resuspension)	25 ml x 1 ea	110 ml x 1 ea	
P2 Buffer (Lysis)	25 ml x 1 ea	110 ml x 1 ea	
PC Buffer (Neutralization)	25 ml x 1 ea	110 ml x 1 ea	
PB Buffer (Binding)	75 ml x 1 ea	180 ml x 2 ea	
WE Buffer (Washing)	120 ml x 1 ea	240 ml x 2 ea	
EA Buffer (Elution)	15 ml x 1 ea	60 ml x 1 ea	
One Page Protocol	1 ea	1 ea	

* Mini – 100 rxn, Midi – 10 rxn, Maxi – 4 rxn

** Mini – 500 rxn, Midi – 50 rxn, Maxi – 18 rxn

Storage

The kit will maintain performance for at least two years under standard storage conditions.

All components of the kit should be stored dry at room temperature (15-25°C). PM1 Buffer is stable for 2 years when store at room temperature. But it must be stored at 4°C after adding lyophilized RNase A. The added RNase A will retain its activity for up to 6 months. RNase A powder can be stored at room temperature for two years. If the P2 Buffer and PB Buffer are stored at lower temperatures for a long time, white precipitates may form. When precipitated, incubate at 60°C until the precipitates are completely dissolved.

Specifications

Scale	8-channel	Mini	Midi	Maxi
Amount of Starting Sample	1-1.5 ml	1-10 ml	20-50 ml	100-200 ml
Turnaround Time	< 5 min	< 5 min	< 10 min	< 15 min
Elution Volume	100 µl	100 µl	500 µl	1 ml
Typical DNA Yield	up to 10 µg	up to 20 µg	up to 200 µg	up to 500 µg
DNA Purity	$A_{260}/A_{280} > 1.8$			
Isolation Technology	Magnetic Nano Bead			

* **Note:** There may be differences in measured values depending on the type of plasmids.

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because PC Buffer and PB Buffer contain chaotropic salts which are irritants.

Introduction

Product Description

MagListo[™] 5M Plasmid Extraction Kit is designed for extraction of highly purified plasmid DNA from cultured bacterial cells. The kit employs Magnetic Nano Beads to extract plasmid DNA with the aid of *MagListo*[™] Magnetic Separation Rack. The use of *MagListo*[™] Magnetic Separation Rack along with the kit greatly increases user convenience by shortening the extraction time without centrifugation. The process does not require phenol/chloroform extraction, or ethanol precipitation.

DNA extracted through this kit can be used for a variety of applications, including: gene cloning, PCR, sequencing, transformation, and *in vitro* transcription/translation.

Principle

Pelleted bacterial cells are resuspended with resuspension buffer containing Nano Beads and lysed under alkaline conditions. Alkaline conditions lead to lysis, release intracellular components, and denature chromosomal, plasmid DNA, and proteins. The resulting lysate is subsequently neutralized in the presence of chaotropic salts. The high salt condition causes denatured proteins, genomic DNA, and cell debris to form insoluble aggregates, while the plasmid DNA renatures in solution. BIONEER's Nano Beads effectively bind to the insoluble aggregates and increases total weight of complexes. The insoluble aggregates are separated using a magnetic force from supernatant containing plasmid DNA, which is then transferred to a new tube for the next process. Guanidine hydrochloride as chaotropic agents in binding buffer removes water molecules around DNA and surface of the silica-coated magnetic nanobeads resulting in plasmid DNA, which is captured by silica-coated magnetic nanobeads. The magnetic nanobeads and DNA complexes are pulled and fixed on the tube wall using a magnetic force. Any salts and precipitates are eliminated by washing buffer, and captured plasmid DNA is eluted in a elution buffer or nuclease-free water.

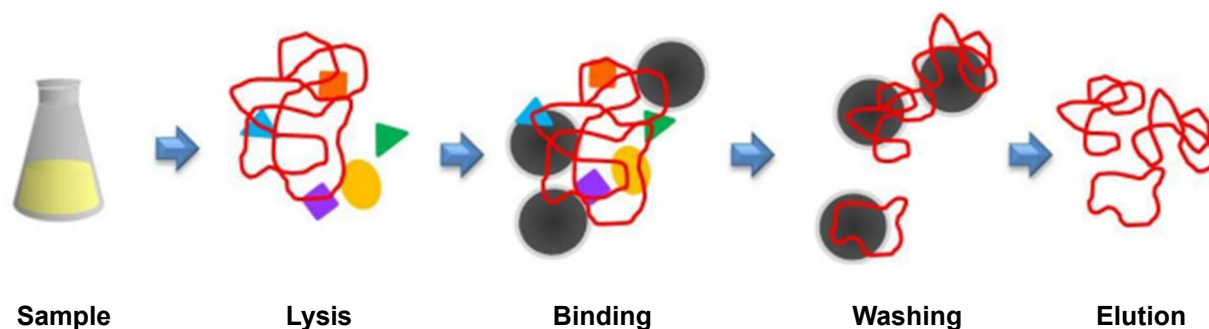


Figure 1. Plasmid DNA extraction using silica-coated magnetic nanobeads.

Features & Benefits

- Convenient: Broad coverage of scales for mini, midi, and maxi isolation protocols with just a single kit (Mini-5 min, Midi-10 min, Maxi-15 min).
- Rapid: Magnetic Nano Beads enable the rapid nucleic acid extraction.
- Efficient: Contaminants such as proteins and nucleases are completely removed.
- Ready-to-use: Extracted DNA is ready-to-use for various application.
- Minimized DNA damage: DNA damage is minimized by avoiding precipitation and use of organic solvents.

Magnetic Nano Beads

Magnetic Nano Beads have been developed to overcome defects of existing resin and to automate purification process. The principle of extraction using the magnetic nanobeads is that coated functional group on the surface of it binds to nucleic acid. Magnetic nanobeads are then isolated using external magnetic field.

Specification

Silica-coated Magnetic Nano Beads	
Matrix	Silica-coated Fe ₃ O ₄
Average size	400 nm
Ligand	- OH
Working Temperature	0-100°C
Storage	Store at room temperature.

Features & Benefits

- **Rapid:** Fast binding guarantees high throughput automation.
- **Effectiveness:** Large surface area enables more sensitive assay.
- **Specificity:** Globular structure increases specificity by decreasing non-specific binding.

MagListo™ Magnetic Separation Rack

MagListo™ Magnetic Separation Rack is designed for a fast and easy separation of the Magnetic Nano Beads. BIONEER offers various racks of different sizes - *MagListo™*-8Ch for 8-tube strip and multi-pipette, *MagListo™*-2 for 2 ml tube, *MagListo™*-15 for 15 ml tube, and *MagListo™*-50 for 50 ml tube. These racks consisting of different size allow user to choose the product according to their needs.

The followings are recommended when handling the *MagListo™* Magnetic Separation Rack.

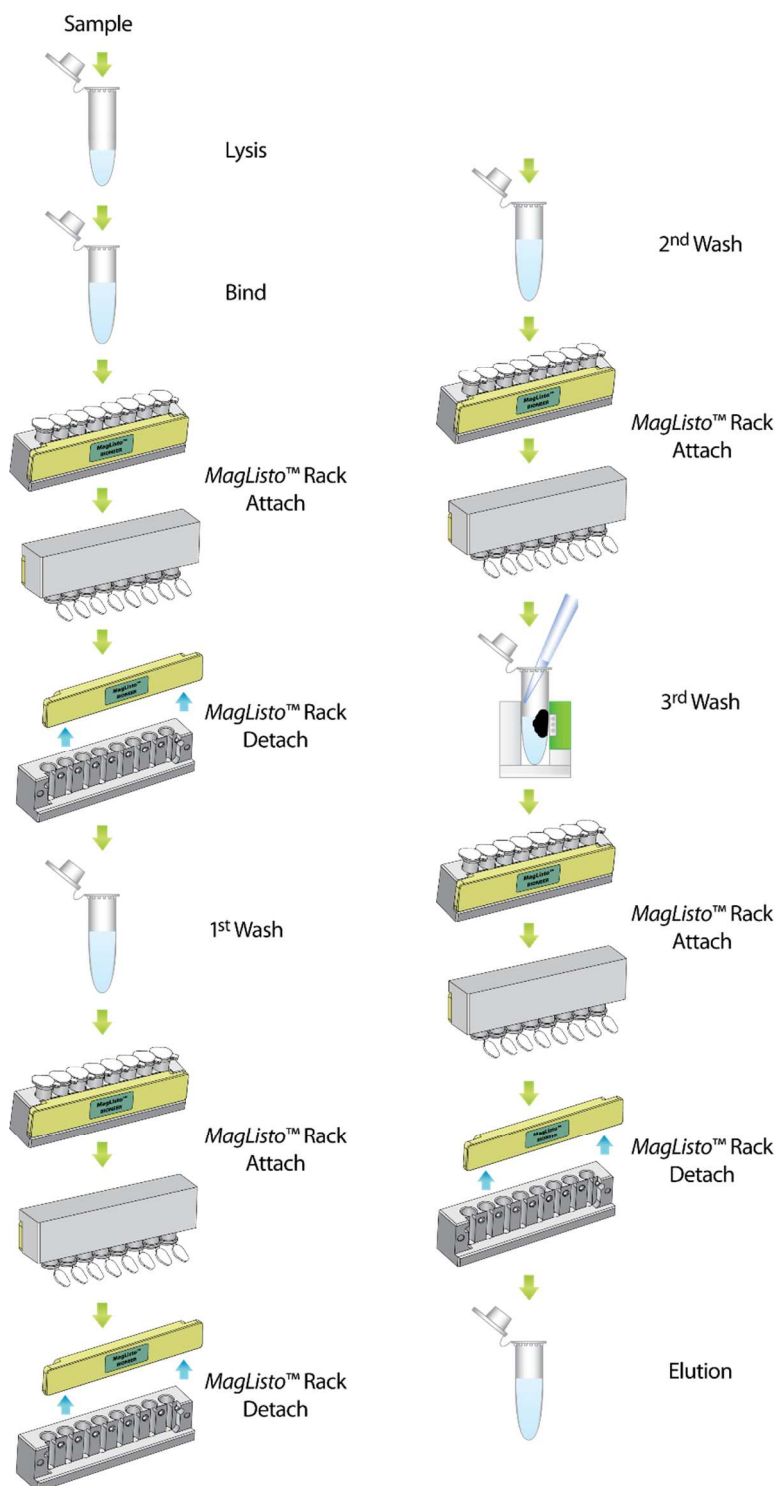
- The product is made of acrylic and plastic. Be careful not to drop the product as the dropping may break the product.
- When moving the product, take extra care not to drop the product as it may cause injury.
- If the product is broken, do not discard it with bare hands as the sharp edges may cause injury.
- When an extracted or purified nucleic acid is spilled on the product, immediately rinse it with running water and clean it with 70% ethanol.
- Acetone, toluene, or organic solvent may cause damage to the acrylic and plastic part of the product, which may lead to malfunction of the product. Rinse the product immediately when the above-mentioned solvent leaks as the expected DNA yield may not be obtained if the product is damaged.
- Check the magnet plate part of the product for corrosive liquid. In the event of a spill, it may corrode the magnet during storage and may deteriorate performance, so rinse immediately with running water.

Features & Benefits

- **Rapid:** Isolates plasmid DNA, genomic DNA, and/or RNA quickly (within 20 minutes) and economically.
- **Convenient:** Simply disposes waste solution just by flipping the rack without centrifugation or pipetting as the silicon fixture secures the tube.

Experimental Procedures

Procedure Overview



Sample Preparation

Several factors such as plasmid copy number, bacterial strain, antibiotics, inoculation and type of culture medium can influence the yield and DNA purity. It is recommended to extract plasmids from bacterial cultures that have been inoculated with a single pure colony from the agar plate.

Copy number of plasmid

Plasmids used in molecular cloning usually contain pMB1 derived replicons (e.g. pUC plasmid). Plasmids containing pMB1 derived replicons have a relaxed control of replication and thus have very high copy numbers. In contrast, pSC101 plasmid has a stringent control of replication and maintain a low copy number. Generally, high copy number plasmids will result in higher yield than low copy number plasmids.

Table 1. Replicons carried by various plasmid vectors.

Plasmids	Copy Number	Replicon	Classification
pUC	500-700	pMB1*	High copy
pGEM	300-500	pMB1*	High copy
pBluescript	300-500	ColE1*	High copy
pTZ	> 1,000	pMB1*	High copy
pET	15-20	pMB1*	Low copy
pAYC	10-12	P15A	Low copy
pBR322	15-20	pMB1*	Low copy
pSC101	> 5	pSC101	Very low copy

* The pMB1 origin of replication (*ori*) is closely related to that of ColE1 and belongs in the same incompatibility group of ColE1. The high copy plasmids listed above contain pMB1 derived replicons.

Host strains

Some strains, especially *endA*⁺ strains such as JM101, JM110, HB101, TG1, and their derivatives may produce lower yield and quality of plasmid DNA than DH1, DH5α, and XL1-Blue strains. In addition, strains derived from HB101 strains can produce large amounts of carbohydrates, which may inhibit activity of many restriction enzymes and polymerases.

Antibiotics

Antibiotics used in bacterial selection can influence growth of bacteria at all stages. As antibiotics are sensitive to storage condition and high temperatures, stock solutions of antibiotics should be stored in aliquots at -20°C. To test whether antibiotics are still effective, fresh cultures inoculated from freshly prepared bacterial culture plates should be used.

Table 2. Recommended concentrations of commonly used antibiotics.

Antibiotics	Stock Solution	Storage	Working Concentration
Ampicillin	40 mg/ml in H ₂ O	-20°C	80 µg/ml
Tetracycline HCl	10 mg/ml in 50% Ethanol	-20°C	50 µg/ml
Kanamycin	10 mg/ml in H ₂ O	-20°C	50 µg/ml

Culture medium

The Luria Bertani (LB) medium is one of the most commonly used media to grow bacterial cells. *MagListo*™ 5M Plasmid Extraction Kit is optimized for use with LB media. As the ratio of plasmid DNA to RNA is higher during the logarithmic phase, it is recommended to collect bacterial cells cultured for approximately 12-16 hours (when stationary growth phase starts).

Before You Begin

Before proceeding, please check the following:

1. Add RNase A powder to PM1 Buffer and completely dissolve it. After adding RNase A powder, PM1 Buffer should be stored at 4°C.

2. Incubate the P2 Buffer and PB Buffer at 60°C when it has precipitates.

3. g-force can be calculated as follows: $rcf = 1.12 \times r \times (rpm/1,000)^2$

* **Note:** Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

Bacterial Culture

- **For 8-channel/mini prep**

1. Pick a single colony from fresh cultured selective plate and inoculate the cells in the 1-5 ml of LB liquid media containing the appropriate selective antibiotics. Incubate at 37°C for 12-16 hours with shaking.

* **Note:** Bacterial overgrowth is not recommended. DNA yields may be reduced because of cell death and inefficient lysis.

- For high-copy number plasmid DNA: 1-5 ml of *E. coli* cells
- For low-copy number plasmid DNA: 2-10 ml of *E. coli* cells

2. Harvest cultured cells by centrifugation at 6,000 x *g* for 15 minutes at 4°C to pellet cells. Discard the supernatant completely using a paper towel by blotting.
3. Proceed immediately to “Plasmid Extraction Using Magnetic Nano Beads” on page 13.

- **For midi/maxi prep**

1. Pick a single colony from fresh cultured selective plate and inoculate the cells in the 5 ml of LB liquid media containing the appropriate selective antibiotics. Incubate at 37°C for 12-16 hours with shaking.
2. Re-inoculate with overnight cultures diluted 1:1,000 in the 20-50 ml (midi)/ 100-200 ml (maxi) of same media as step 1. Incubate at 37°C for 12-16 hours with shaking.
3. Harvest cultured cells by centrifugation at 6,000 x *g* for 15 minutes at 4°C to pellet cells. Discard the supernatant completely using a paper towel by blotting.
4. Proceed immediately to “Plasmid Extraction Using Magnetic Nano Beads” on page 13.

Plasmid Extraction Using Magnetic Nano Beads

1. **(Resuspension)** Resuspend the cell pellet in 200 μ l (mini)/ 2 ml (midi)/ 6 ml (maxi)/ 100 μ l (8-channel) of PM1[†] Buffer by vortexing or pipetting.

* **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency. If you have harvested cell pellet in other size of tubes, please transfer the resuspended cells to a 1.5 ml or 2 ml (mini)/ 15 ml (midi)/ 50 ml (maxi)/ 1 ml (8-channel) tube.

[†] PM1 Buffer contains Magnetic Nano Beads, please shake well before use. Incomplete suspension can lead to partial removal of aggregation.

2. **(Lysis)** Add 200 μ l (mini)/ 2 ml (midi)/ 6 ml (maxi)/ 100 μ l (8-channel) of P2 Buffer to the sample and mix gently by inverting the tube 3-4 times.

* **Note:** Vortexing should be avoided because this will cause shearing of genomic DNA and contamination of plasmid DNA with gDNA.

3. **(Neutralization)** Add 200 μ l (mini)/ 2 ml (midi)/ 6 ml (maxi)/ 100 μ l (8-channel) of PC Buffer and mix immediately by inverting the tube 3-4 times.

* **Note:** Genomic DNA and cell debris will form an insoluble complex with magnetic nanobeads in this step. Be cautious not to shear genomic DNA.

4. **(Debris removal)** Place the tube in *MagListo*[™]-2 (mini)/ -15 (midi)/ -50 (maxi)/ -8Ch (8-channel) Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

* **Note: (Optional)** For better yield, centrifuge the tube at 13,000 rpm for 1 min (mini, 8-channel)/ 5 min (midi)/ 10 min (maxi). Transfer cleared lysate to a new 1.5 ml or 2 ml (mini)/ 15 ml (midi)/ 50 ml (maxi) tube and go to step 7.

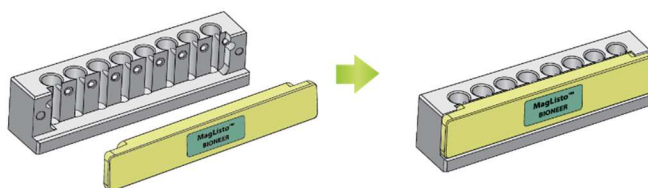


Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

5. Without removing the tube from *MagListo*[™] Magnetic Separation Rack, transfer the supernatant carefully to a new 1.5 ml or 2 ml (mini)/ 15 ml (midi)/ 50 ml (maxi)/ 1 ml (8-channel) tube.

6. Repeat steps 4-5 for clearer supernatant.
7. **(Plasmid binding)** Add 600 µl (mini)/ 6 ml (midi)/ 18 ml (maxi)/ 300 µl (8-channel) of PB Buffer and then add 75 µl (mini)/ 450 µl (midi)/ 2 ml (maxi)/ 35 µl (8-channel) of evenly mixed Magnetic Nano Bead solution to each tube. Close the cap and invert the rack 3-4 times with the magnet plate detached.
8. Place the tube in *MagListo*™ Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.
9. Without removing the tube from *MagListo*™ Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting. During this process, the beads containing DNA remain attached to the side of the tube.

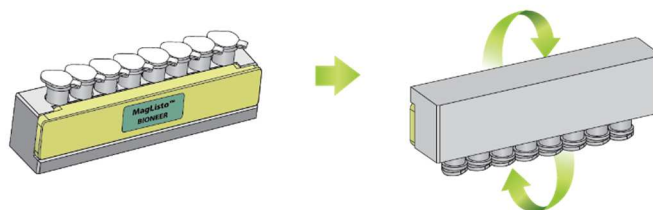


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™-2 Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

10. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 1 ml (mini)/ 10 ml (midi)/ 30 ml (maxi)/ 500 µl (8-channel) of 80% ethanol (not provided) to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

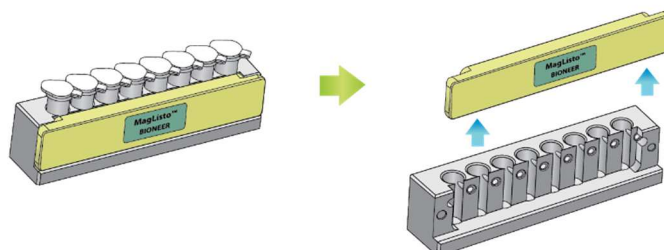


Figure 4. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

11. Attach the magnet plate to *MagListo*[™] stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.
12. Without removing the tubes from *MagListo*[™] Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.
13. **(2nd Washing)** Repeat steps 10-12 by adding 1 ml (mini)/ 10 ml (midi)/ 30 ml (maxi)/ 500 µl (8-channel) of 80% ethanol (not provided) for additional washing.
14. **(3rd Washing)** Without removing the tubes from *MagListo*[™] Magnetic Separation Rack, add 900 µl (mini)/ 10 ml (midi)/ 25 ml (maxi)/ 700 µl (8-channel) of WE Buffer to the opposite side of beads. Close the cap and invert the rack twice in order to remove ethanol from the sample.
15. Discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

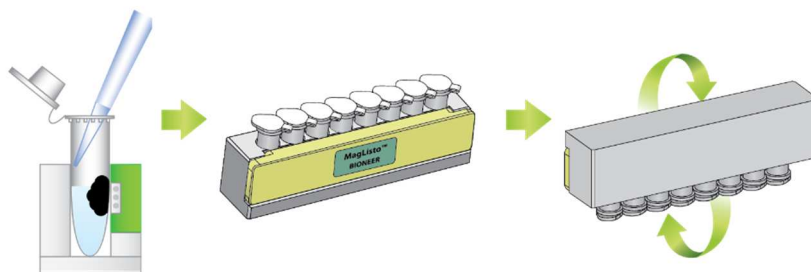


Figure 5. Washing the beads to remove residual ethanol. Please refer to the information above.

* **Note:** Direct pipetting of WE Buffer onto the beads, vortexing and/or vigorous shaking of the tubes may release nucleic acid from the beads, which may result in lower DNA yield.

16. **(Elution)** Detach the magnet plate from *MagListo*[™] Magnetic Separation Rack. Add 100 µl (mini, 8-channel)/ 500 µl (midi)/ 1 ml (maxi) of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.
17. Incubate at 60°C for 1 minute.

18. Attach the magnet plate to *MagListo*™ Magnetic Separation Rack and invert the rack gently 3-4 times until the beads bind tightly to the magnet.
19. Without removing the tube from *MagListo*™ Magnetic Separation Rack, transfer supernatant containing DNA carefully to a new tube.
20. Discard the tubes with the remaining beads.

* **Note:** Do not reuse the beads.

Summary of Reagent Volumes Required in Each Step of Plasmid Extraction

Step	Buffer	8-channel	Mini	Midi	Maxi
Sample collection	Culture volume	1-1.5 ml	1-10 ml	20-50 ml	100-200 ml
Resuspension	PM1 Buffer	100 µl	200 µl	2 ml	6 ml
Lysis	P2 Buffer	100 µl	200 µl	2 ml	6 ml
Neutralization	PC Buffer	100 µl	200 µl	2 ml	6 ml
Plasmid Binding	PB Buffer	300 µl	600 µl	6 ml	18 ml
Bead Binding	Magnetic Nano Bead	35 µl	75 µl	450 µl	2 ml
1 st , 2 nd Washing	80% Ethanol	500 µl	1 ml	10 ml	30 ml
3 rd Washing	WE Buffer	700 µl	900 µl	10 ml	25 ml
Elution	EA Buffer	< 100 µl	< 100 µl	< 500 µl	< 1 ml

Troubleshooting

Problem	Comments
Low plasmid DNA yield	<ul style="list-style-type: none"> • Buffers or other reagents may have been exposed to conditions that reduce their effectiveness. Store the reagents at room temperature (15-25°C) at all times upon arrival and keep all reagent bottles tightly closed after use to preserve pH and stability and avoid contamination.
	<ul style="list-style-type: none"> • Cell debris may not have been fully removed. Proceed debris removal step, especially for mini or 8-channel scale. For more information, refer to step 4 (optional) on page 13.
	<ul style="list-style-type: none"> • You may have used too much culture. Too much culture causes incomplete lysis and neutralization. For more information, refer to “Specifications” on page 2.
	<ul style="list-style-type: none"> • The cells may not have been completely resuspended with PM1 Buffer. Resuspend completely by vortexing or pipetting.
	<ul style="list-style-type: none"> • Elution may have been incomplete. Extend incubation time up to 3 minutes at elution step to improve the yield. In addition, make sure that Magnetic Nano Beads are suspended completely in the Elution Buffer during incubation.
	<ul style="list-style-type: none"> • Insufficient shaking or vortexing during lysis step may lead to low DNA yield. Shake or mix by vortexing sufficiently during incubation step.

	<ul style="list-style-type: none"> • Pellet of Magnetic Nano Beads could be lost while discarding solution. Check that all of magnetic nanobeads bind tightly to magnet when you discard solution. • PM1 Buffer may have been over 6 months since you added RNase A powder. If it has been over 6 months since adding the RNase A powder to the PM1 Buffer, the RNase A may not work properly. Add more RNase A powder up to 100 ng/μl.
Low A_{260/280} ratio	<ul style="list-style-type: none"> • Magnetic Nano Beads may have been washed insufficiently. Wash the beads properly in the 3rd washing step. Remaining ethanol can decrease the DNA purity. Take enough time to properly wash the beads. • Incomplete suspension of Magnetic Nano Beads during the washing step causes salts to remain in the purified DNA. Make sure that the beads are suspended thoroughly during the washing step.
Appearance of unexpected bands following gel electrophoresis	<ul style="list-style-type: none"> • There may have been contamination of chromosomal DNA. Avoid vortexing the samples vigorously during neutralization step. Lysis time should not exceed 5 minutes. Both will result in shearing of genomic DNA. So, handle the lysate gently.
Aggregation of Magnetic Nano Beads	<ul style="list-style-type: none"> • You may have used too much culture. Add appropriate amount of culture. For more information, refer to “Specifications” on page 2.
Presence of a white precipitates in some buffers	<ul style="list-style-type: none"> • PB Buffer may have been stored at lower temperatures for a long time. If precipitated, incubate at 60°C to dissolve any precipitates in

	the buffer.
Degraded DNA	<ul style="list-style-type: none"> • Repeated freezing and thawing may degrade DNA. Avoid repeated freezing and thawing.
Sample floating upon loading in an agarose gel	<ul style="list-style-type: none"> • Sample may contain ethanol. Floating is caused by remaining ethanol. Ensure that the 3rd washing (ethanol removing) step in the protocol is properly performed.
Appearance of too many background bands in sequencing analysis	Check the endonuclease activity of your host <i>E. coli</i> strain. HB101, JM series, and normal wild-type hosts that have high endonuclease activity interrupt the sequencing reaction by degrading the plasmid. We recommend using the <i>endA</i> ⁻ strain instead of <i>endA</i> ⁺ strain.

References

Bonham, M. J., & Danielpour, D. (1996). Improved purification and yields of RNA by RNeasy®. *Biotechniques*, 21(1), 57-60.

Coombs, N.J., Gough, A.C., and Primrose J.N. (1999) Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. *Nucleic Acids Research*, 27(16), e12.

Reno, C., Marchuk, L., Sciore, P., Frank, C.B., and Hart, D.A. (1997) Rapid isolation of total RNA from small samples of hypocellular, dense connective tissues. *Biotechniques*, 22(6), 1082-1086.












Ordering Information

Description		Cat. No
<i>MagListo</i> ™ 5M Plasmid Extraction Kit	100 reactions	K-3601
	500 reactions	K-3600

Related Products

Description	Cat. No
RNase A Powder	KB-0101
<i>MagListo</i> ™-8Ch Magnetic Separation Rack	TM-1000
<i>MagListo</i> ™-2 Magnetic Separation Rack	TM-1010
<i>MagListo</i> ™-2-12h Magnetic Separation Rack	TM-1011
<i>MagListo</i> ™-15 Magnetic Separation Rack	TM-1020
<i>MagListo</i> ™-50 Magnetic Separation Rack	TM-1030

Explanation of Symbols

 Batch Code	 Consult Instructions For Use	 Research Use Only	 Caution
 Biological Risks	 Contains Sufficient for <n> tests	 Temperature Limitation	 Manufacturer
 Catalog Number	 Do not Re-use	 Use-by Date	

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