USER GUIDE

MagListo™ 5M PCR/Gel Purification Kit

Cat. No. K-3627

BIONEER
Innovation • Value • Discovery

MagListo™ 5M PCR/Gel Purification Kit

Kit for the purification of fragment DNA from PCR products or agarose gel

User Guide

K-3627

Σ 100

Version No.: 0 (2022-03-24)

Please read all the information in booklet before using the unit

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Intended Use

MagListo™ 5M PCR/Gel Purification 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).

Warranty and Liability

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.

Trademark

MagListo™ is a trademark of BIONEER Corporation.

Copyright

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

This kit contains adequate reagents for 100 reactions.

Components	Amount*	Storage
Magnetic Nano Bead	11 ml x 1 ea	Store at room temperature (15-35°C).
PB Buffer (Binding)	50 ml x 1 ea	
FB Buffer (Binding)	120 ml x 1 ea	
FWM1 Buffer (1st Washing)	80 ml x 1 ea	
W2 Buffer (2nd Washing)	80 ml x 1 ea	
EA Buffer (Elution)	25 ml x 1 ea	
One Page Protocol	1 ea	

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 (15-35°C).

Specifications

Sample Type	PCR product	Agarose gel
Size range	100 bp – 10 Kb	
Typical recovery	≥ 80%	≥ 50%
Expected purity	A_{260}/A_{280} > 1.8	
Extraction time	< 5 min	< 15 min

*** Note:** Gels containing ≤1% agarose for the gel purification is required. The high contents of the agarose gel will affect to the DNA purity and recovery.

Purification of fragment DNA from PCR product and agarose gel

MagListo™ 5M PCR/Gel Purification Kit can extract DNA from PCR product and agarose gel, respectively. For PCR product, the optimal size range is from 100 bp to 10 Kb. In the binding step, PB buffer should be added 5 times the PCR product volume. After mixing well, use the *MagListo*™ Magnetic Separation Rack to perform subsequent steps.

In the case of gel-electrophoresis, put a piece of gel in a tube to measure its weight. And for binding step, add FB buffer which is three times the weight of that gel. Incubate the tube at 60℃ for 10 minutes and vortex it every 2-3 minutes.

Recommended amounts of starting sample

It is recommended to use the amounts in Table 1 as starting sample amount.

Table 1. Optimal volume and recommended products according to sample type.

Sample type	Sample volume	Recommended BIONEER's products
PCR Product		
-	20 ul/tube	K-2012, K-2016 K-2601, K-2602 K-2022, K-2024, K-2631, etc.
-	50 ul/tube	K-2013, K-2017, K-2011 K-2603, K-2604 K-2023, K-2025, K-2027 K-2633, etc.
Mix the multiple PCR products	~ 200 ul/tube	-
Agarose gel		
TAE Buffer (C-9004), TBE Buffer (C-9002)	~ 400 mg/tube	A-7020, A-7020-3, A-7020-4 A-6020, C-9100, C-9036

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because PB Buffer, FB Buffer, FWM1 Buffer, W2 Buffer contain chaotropic salts, 2-Propanol and ethanol which are irritants.
- Thoroughly mix PB Buffer (add Isopropanol) by shaking before use.

Introduction

Product Description

MagListo™ 5M PCR/Gel Purification Kit is designed for purification of highly purified fragment DNA from PCR product and agarose gel. The kit employs Magnetic Nano Beads to extract nucleic acid with the aid of *MagListo*™ Magnetic Separation Rack and *ExiPrep*™ 96 Lite (Cat. No. A-5250). The use of *MagListo*™ Magnetic Separation Rack along with the kit greatly increases user convenience by shortening the extraction time without centrifugation. On the same principle, *ExiPrep*™ 96 Lite is designed for rapid extraction of nucleic acids delivering up to 96 extracted samples (including controls).

Purified nucleic acid through this kit can be used for a variety of applications, including gene cloning, subcloning, sequencing, labeling, DNA concentration and other molecular biological applications.

Principle

1. PCR Purification

MagListo™ 5M PCR/Gel Purification Kit is designed for the purification of PCR product from PCR mixture and enzymatic products within 5 min. The overall principle is based on adsorption of DNA onto the silica coated magnetic nano bead by chaotropic salt. For instance, PB Buffer (PCR Binding) contains chaotropic reagent like guanidine hydrochloride, which removes water molecules around DNA and magnetic nano bead surface resulting in fragment DNA then being captured by magnetic beads. The magnetic nano bead and nucleic acid complexes are pulled and fixed on the tube wall using a magnetic force, followed by washing with ethanol to remove debris and excessive salts. The captured nucleic acids are then eluted by EA Buffer (Elution), an aqueous solution with optimal pH.

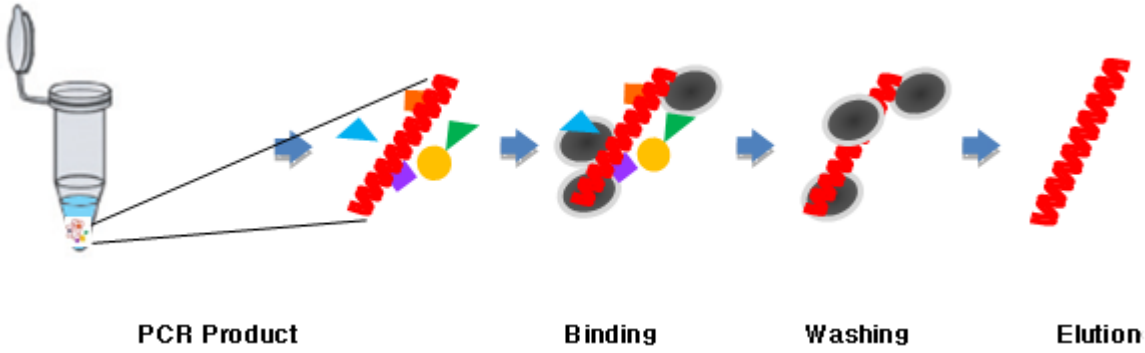


Figure 1. PCR product purification using silica-coated magnetic nanobeads.

2. Gel Purification

MagListo™ 5M PCR/Gel Purification Kit is designed for the extraction of highly purified fragment DNA from agarose gel with 1X TAE or TBE buffer within 15 min. The overall principle is based on adsorption of DNA onto the silica coated magnetic nano bead by chaotropic and other salt components which also enhance melting of agarose gel. For example, FB Buffer (Gel Solubilization) contains chaotropic component such as guanidine thiocyanate and removes water molecules around DNA and magnetic nano bead surface resulting in fragment DNA then being captured by magnetic beads. The magnetic nano bead and nucleic acid complexes are pulled and fixed on the tube wall using a magnetic force, followed by washing with ethanol to remove debris and excessive salts. The captured nucleic acids are then eluted by EA Buffer (Elution), an aqueous solution with optimal pH.

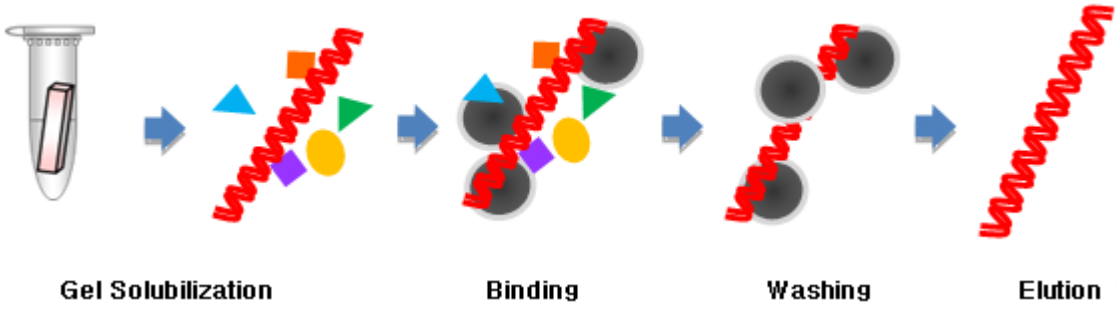


Figure 2. Agarose gel purification using silica-coated magnetic nanobeads.

Features & Benefits

- Comprehensive: High quality and yield of nucleic acid from various samples such as PCR product or agarose gel.
- Convenient: Broad coverage of scales for PCR product or agarose gel with just a single kit.
- Rapid: Magnetic Nano Beads enable the rapid nucleic acid purification.
- Efficient: A wide range of possible sample size (100 bp ~ 10 Kb)
- Cost-effective: Can be applied to *ExiPrep*™ 96 Lite to automate PCR/Gel purification

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_3O_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 muti-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.

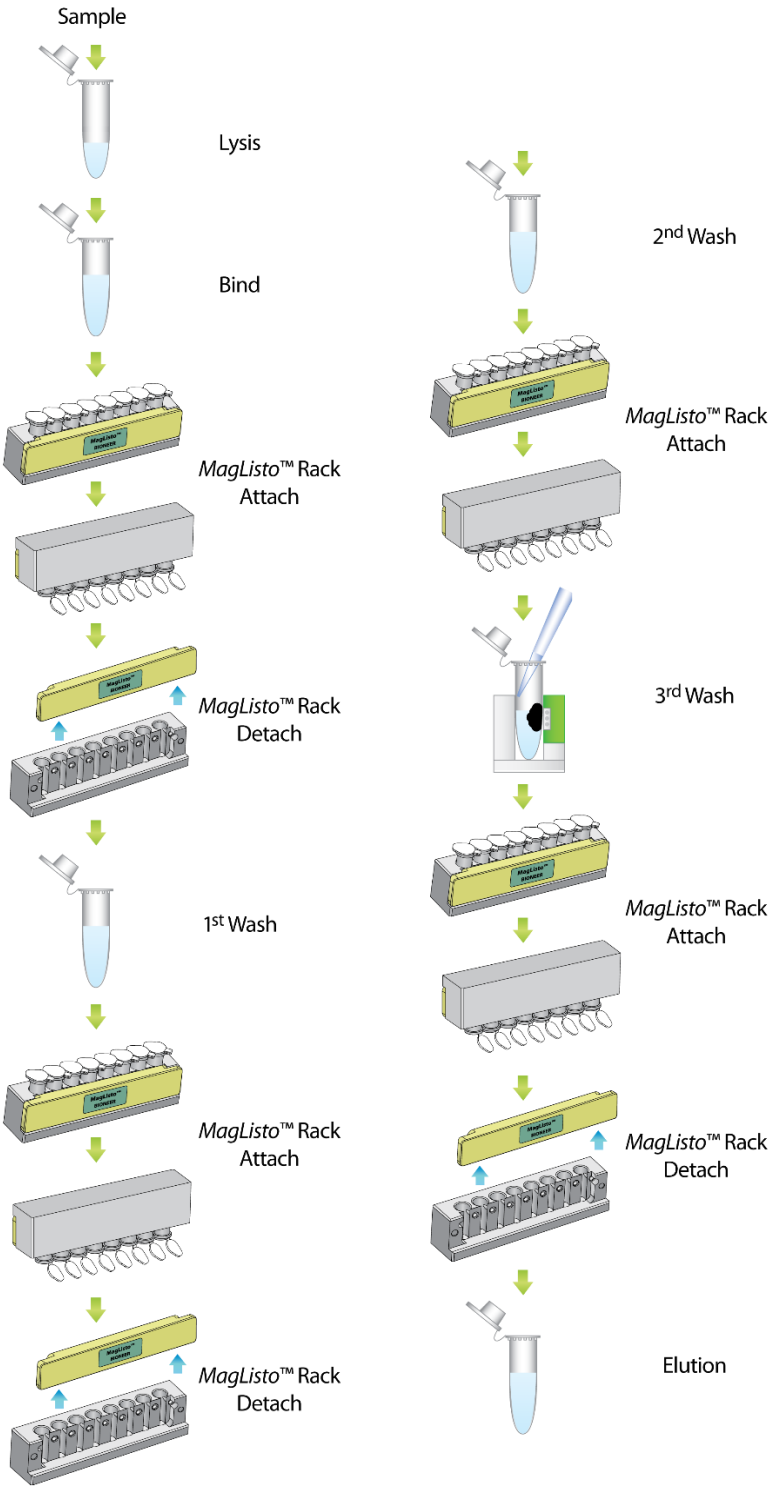
- The product is made of acryl and plastic. Be careful not to drop the product as the dropping may break the product.
- When moving the product, take extra care not to drop the product as it may cause injury.
- If the product is broken, do not discard it with bare hands as the sharp edges may cause injury.
- 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 sill, 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 harvesting method and storage of starting samples can influence the yield and nucleic acid purity. All samples must be stored in a freezer or used immediately after collection. It is recommended to put the sample as soon as possible on ice and avoid repeated freezing and thawing.

PCR product

PCR product can easily be got by AllInOneCycler™ PCR system (A-2041). PCR product should immediately be used or stored in a freezer. Sample can be stored for several days at 4°C and for up to 1 year at -70°C. It is recommended to defrost the sample rapidly or store it on ice before use.

Agarose gel

Agarose gel sample can be processed in an electrophoresis. When making the agarose gel, it is recommended to have a concentration of less than 1%. After electrophoresis, carefully transfer the agarose gel to the DUALED Blue/White Transilluminator (A-6020) and check the position of the target band. If DNA cloning is the purpose, minimize UV exposure time. Then, Cut the target band with a knife and put it in an empty micro-centrifuge tube. Measure and record the weight of the tube containing gel. It is recommended to proceed with the gel solubilization step immediately using the FB Buffer, and if it is not possible, refrigerated storage (4°C) can be temporarily performed within 1 hour.

Before You Begin

Before proceeding, please check the following:

1. Prepare the absolute isopropanol and absolute ethanol (not provided).
2. Gels containing less than 1% agarose concentration for the purification are required.
3. Add indicated volume of absolute isopropanol (not provided) to PB Buffer before use (see bottle label).
4. Incubate the FB Buffer at 60°C when it has precipitates.

PCR Purification from PCR product

1. Transfer the PCR product to the clean tube.

2. **(Binding)** Add 5 volumes of PB Buffer to the PCR product. Mix well using a vortex mixer or by pipetting
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.
   * **Note:** For 20 μl of sample, add 100 μl of PB Buffer in micro-centrifuge tube.

3. **(Binding with Magnetic Nano Bead)** Add 100 μl of Magnetic Nano Bead solution to the tube. Mix completely by vortex mixer or pipetting until the beads are fully resuspended.
   * **Note:** Magnetic Nano Bead solution contains magnetic nano beads. Please shake well or mix with a vortex mixer before use.

4. Place the tube in *MagListo*™-2 or *MagListo*™-15 Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

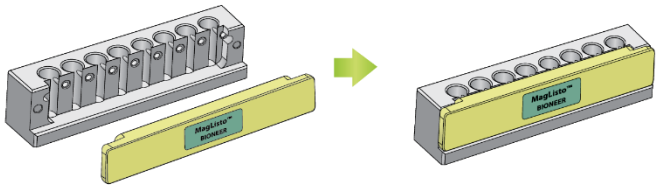


Figure 1. Attachment of the magnet plate. Combine the magnet plate to the stand.

5. 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.

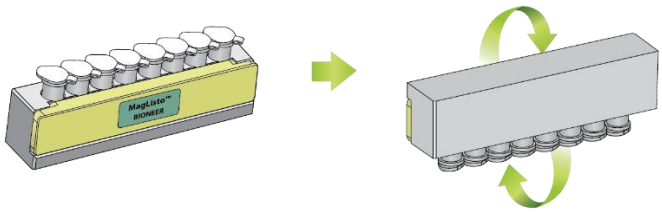


Figure 2. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the

solution does not spill on the rack.

6. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 700 µl of W2 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

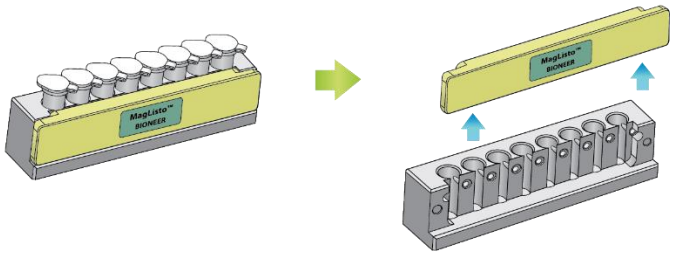


Figure 3. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

7. Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

8. 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.

9. **(2nd Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Repeat steps 7-8 by adding 700 µl of absolute ethanol for additional washing. Repeat steps 7-8 once more.

10. **(Drying)** Detach the magnet plate from the stand. Completely dry the beads with the tube opened at 60°C using the Heating Block for 10 min. Remove the remaining supernatant using the pipette.

11. **(Elution)** Add 30 µl ~ 50 µl of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.

12. Incubate tubes at 60°C for more than 1 minute.

13. 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.

14. Without removing the tube from *MagListo*™ Magnetic Separation Rack, transfer supernatant containing DNA carefully to a new tube.

15. Discard the tubes with the remaining beads.

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

Gel Purification from Agarose gel

1. **(Gel Cutting)** Visualize the band, in a red-safe stained gel, in a dark room with an UV light. Cut around the band of interest DNA using a scalpel blade. Switch off the UV light-box, carefully remove the slice from the gel and weigh the gel slice in a 1.5 ml or 2 ml tube (not provided).
   * **Note:** Set the UV light-box to long wave length UV, if possible, and minimize the exposure time to DNA.

2. **(Gel Solubilization)** The maximum amount of gel slice per each sample is ~ 400 mg. Add 3 volumes of FB Buffer to the 1 volume of the gel slice.
   * **Note:** If the weight of gel slice is 200 mg, add 600 µl of FB Buffer.

3. Incubate at 60℃ for 10 min. Mix by inverting the tube every 2-3 min during the incubation.
   * **Note:** If the gel slice is dissolved incompletely, increase the incubation time. After dissolving the gel slice, check the color of the mixture whether it is yellow or not. If the color of the mixture is orange or red, add 10 µl of 3 M sodium acetate (pH 5.0) and mix so that the color should be turned into yellow.
   * **Note:** The color of the mixture indicates pH of the mixture related with DNA binding. pH ≤ 7.5 (yellow color), the fragment DNA can effectively bind to the magnetic nano beads.

4. **(Binding with Magnetic Nano Bead)** Add 100 µl of Magnetic Nano Bead solution to the tube. Mix completely by vortex mixer or pipetting until the beads are fully resuspended.
   * **Note:** Magnetic Nano Bead solution contains magnetic nano beads. Please shake well or mix with a vortex mixer before use.

5. Place the tube in *MagListo*™-2 or *MagListo*™-15 Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

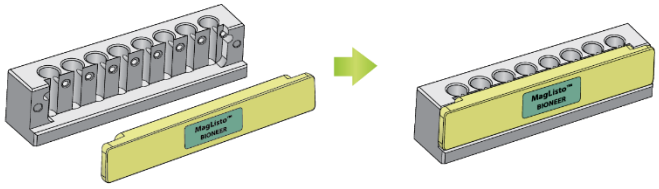


Figure 1. Attachment of the magnet plate. Combine the magnet plate to the stand.

6. 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.

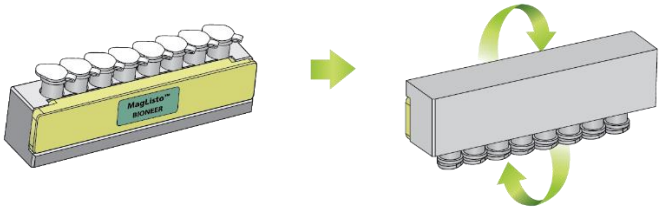


Figure 2. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

7. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 700 µl of FWM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

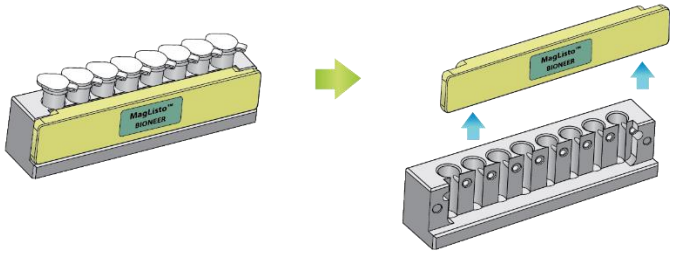


Figure 3. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

8. Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

9. 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.

10. **(2nd Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Repeat steps 8-9 by adding 700 µl of W2 Buffer.

11. **(3rd washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Repeat steps 8-9 by adding 700 µl of Absolute ethanol.

12. **(Drying)** Detach the magnet plate from the stand. Completely dry the beads with the tube opened at 60°C using the Heating Block for 10 min. Remove the remaining supernatant using the pipette.

13. **(Elution)** Add 30 µl ~ 50 µl of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.

14. Incubate tubes at 60°C for more than 1 minute.

15. 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.

16. Without removing the tube from *MagListo*™ Magnetic Separation Rack, transfer supernatant containing DNA carefully to a new tube.

17. Discard the tubes with the remaining beads.

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

Troubleshooting

PCR Purification

Problem	Comments
Low recovery of fragment DNA	• **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.
	• **Excess amount of starting sample was used to extract DNA.** Appropriate amount of starting sample (see "Specification" in page 3) should be used for efficient purification of PCR product.
	• **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.
	• **Insufficient shaking or vortexing during lysis step may lead to low DNA yield.** Shake or mix by vortexing sufficiently during incubation step.
Low $A_{260/280}$ ratio	• **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.
Aggregation of Magnetic Nano Beads	• **You may have used too much starting material.** Add appropriate amount of starting material. For more information, refer to “Specifications” on page 2.
Presence of a white precipitates in some buffers	• **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.
Sample floating upon loading in an agarose gel	• **Sample may contain ethanol.** Floating is caused by remaining ethanol. Ensure that the drying (ethanol removing) step in the protocol is properly performed.

Gel Purification

Problem	Comments
Low recovery of fragment DNA	• **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.
	• **High pH conditions can reduce the overall yield due to incorrect binding conditions.** This can be examined by using FB Buffer (Gel Solubilization), which has a pH indicator. If the color is yellow but it turns to red or orange, then the pH is out of range. In this case, several drops of sodium acetate buffer are recommended to adjust the pH of the solution appropriately.
	• **Incomplete dissolving DNA of the gel slices gives lower yield.** DNA can be remained in any undissolved agarose gel. Provide enough time for gel slice to be dissolved completely.
	• **Some of Magnetic Nano Bead pellet may have been lost while discarding solution** Check that all of the Nano Beads have bound tightly to the magnet when you discard supernatant.
	• **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.

	• **Insufficient shaking or vortexing during lysis step may lead to low DNA yield.** Shake or mix by vortexing sufficiently during incubation step.
Low $A_{260/280}$ ratio	• **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.
Aggregation of Magnetic Nano Beads	• **You may have used too much starting material.** Add appropriate amount of starting material. For more information, refer to “Specifications” on page 2.
Presence of a white precipitates in some buffers	• **FB 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.
Sample floating upon loading in an agarose gel	• **Sample may contain ethanol.** Floating is caused by remaining ethanol. Ensure that the drying (ethanol removing) step in the protocol is properly performed.

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
MagListo™ 5M PCR/Gel Purification Kit	100 reactions	K-3627

Related Products

Description	Cat. No
MagListo™-8Ch Magnetic Separation Rack	TM-1000
MagListo™-2 Magnetic Separation Rack	TM-1010
MagListo™-2-12h Magnetic Separation Rack	TM-1011
MagListo™-15 Magnetic Separation Rack	TM-1020
MagListo™-50 Magnetic Separation Rack	TM-1030

Explanation of Symbols

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

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