USER GUIDE

MagListo™ cfDNA Extraction Kit

Cat. No. K-3619

BIONEER
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MagListo™ cfDNA Extraction Kit

Kit for the extraction of cfDNA from plasma, serum, urine, and saliva

User Guide

K-3619

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Version No.: 5 (2025-02-10)

Please read all the information in booklet before using the unit

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Intended Use

MagListo™ cfDNA 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).

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

Components	Amount*	Storage
Proteinase K powder, lyophilized	25 mg x 2 ea	Refer to the “Storage” below.
Magnetic Nano Bead	11 ml x 1 ea	Store at room temperature (15-25°C).
CL Buffer (Lysis)	110 ml x 1 ea	
CB Buffer (1st Washing)	110 ml x 1 ea	
CW1 Buffer (2nd Washing)	110 ml x 1 ea	
TA Buffer (3rd Washing)	120 ml x 1 ea	
EA Buffer (Elution)	25 ml x 1 ea	
One Page Protocol	1 ea	

* Mini – 50 rxn, Midi – 16 rxn, Maxi – 10 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). Before use, lyophilized Proteinase K should be completely dissolved in 1,250 µl of nuclease-free water. For short term storage, dissolved Proteinase K should be stored at 4°C. For long term storage, it is recommended to aliquot these enzymes into separate tubes and store at -20°C.

*** Note:** Please note that repeated freezing and thawing may reduce its activity.

Specifications

MagListo™ cfDNA Extraction Kit			
Sample Type	**Mini**	**Midi**	**Maxi**
Plasma/Serum/Urine/Saliva	< 1 ml	< 3 ml	< 5 ml
Elution Volume	30 µl	50 µl	70 µl
Typical DNA Yield	up to 5 ng	up to 15 ng	up to 25 ng
DNA Purity	A_{260}/A_{280} > 1.8		

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

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because CL Buffer, CB Buffer and CW1 Buffer contain chaotropic salts which are irritants.

Introduction

Product Description

MagListo™ cfDNA Extraction Kit is designed for extraction of highly purified cfDNA from plasma, serum, urine, and saliva. The kit employs Magnetic Nano Beads to extract cfDNA 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). In the case of *MagListo*™ cfDNA Extraction Kit, up to 24 extracted samples can be delivered by using *ExiPrep*™ 96 Lite. The process does not require phenol/chloroform extraction and ethanol precipitation.

DNA extracted through this kit can be used for a variety of applications, including: PCR, Real-time PCR, digital PCR, and Next-Generation Sequencing (NGS).

Principle

MagListo™ cfDNA Extraction Kit is designed for extraction of circulating cell free DNA from plasma, serum, urine, and saliva. The kit employs Magnetic Nano Beads coated with silica for nucleic acid binding in the presence of chaotropic salts. Guanidine hydrochloride as chaotropic agents in binding buffer removes water molecules around DNA and surface of the silica-coated magnetic nanobeads resulting in 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 DNA is eluted in an elution buffer or nuclease-free water.

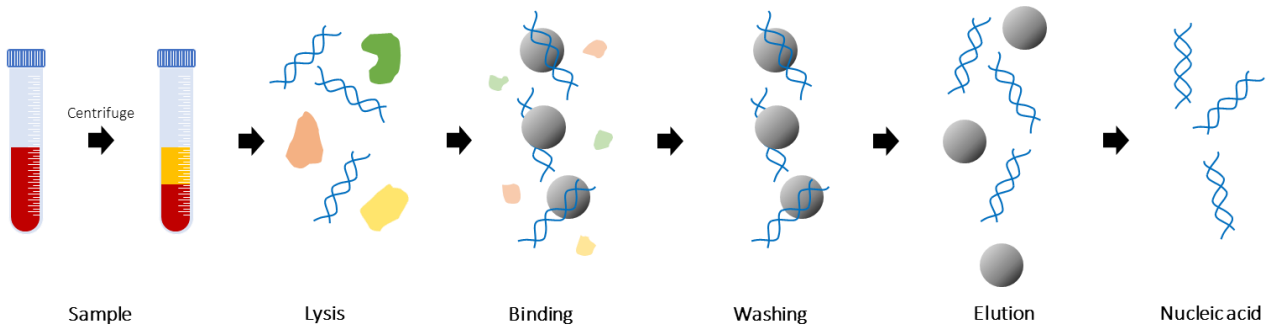


Figure 1. cfDNA extraction using silica-coated magnetic nanobeads.

Features & Benefits

- Comprehensive: High quality and yield of genomic DNA extraction from various samples such as plasma, serum, urine, or saliva.
- Rapid: Magnetic Nano Beads enable the rapid nucleic acid extraction.
- Convenient: Broad coverage of scales for mini, midi, and maxi isolation protocols with just a single kit.
- Cost-effective: Can be applied to *ExiPrep*™ 96 Lite to automate cfDNA extraction.

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*™-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.

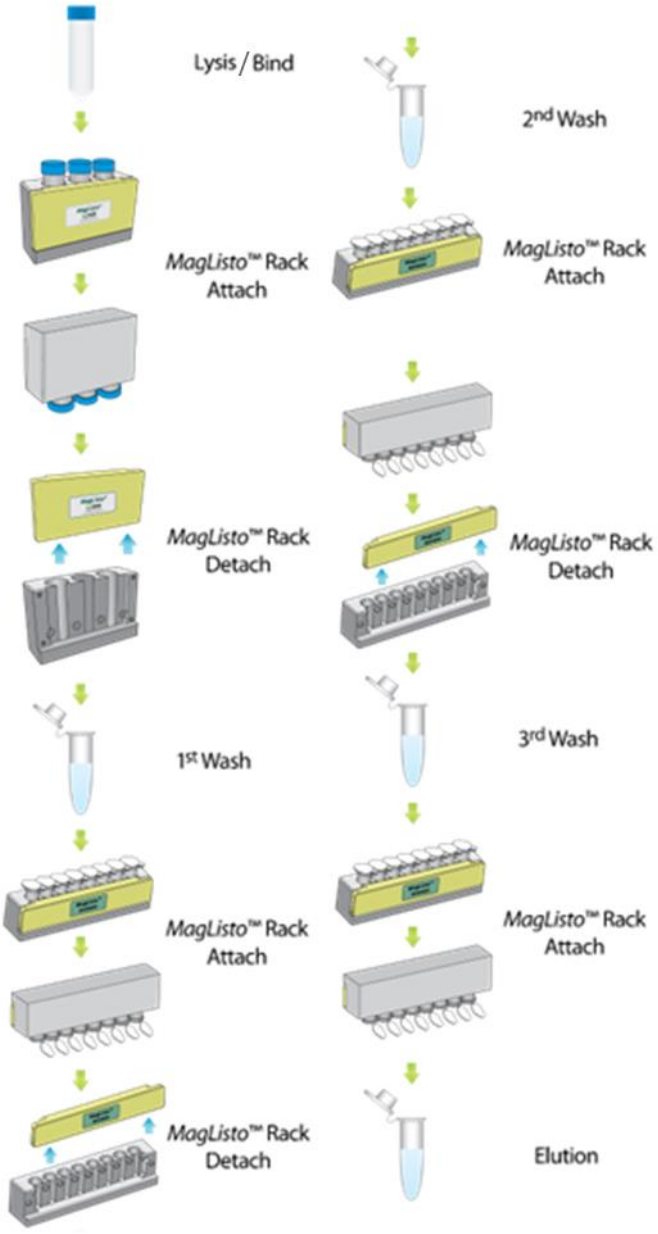
- The product is made of acryl and plastic. Be careful not to drop the product as the dropping may break the product.
- When moving the product, take extra care not to drop the product as it may cause injury.
- If the product is broken, do not discard it with bare hands as the sharp edges may cause injury.
- 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 cfDNA purity. All specimens 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.

Plasma

Plasma should be collected in a tube containing anticoagulants for blood (EDTA and ACDs). 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 in a water bath (37°C) and store it on ice before use.

Serum

Serum should be collected in a tube containing a serum separator (SST Tube). Sample can be stored for 7 days at 4°C and for up to 1 year at -70°C. It is recommended to defrost the sample rapidly in a water bath (37°C) and store it on ice before use.

Urine

Urine sample should immediately be used or stored in a collection tube. It is recommended to collect first morning urine sample. Sample can be stored for up to 7 days at 4°C.

Saliva

Saliva sample should immediately be used or stored in a collection tube. Sample can be stored for up to 7 days at 4°C.

Before You Begin

Before proceeding, please check the following:

1. Completely dissolve Proteinase K powder in 1,250 µl of nuclease-free water before use. Dissolved Proteinase K should be stored at 4°C.

2. Preheat EA Buffer to 60°C before use. This step is essential for optimal recovery during magnetic bead-based extraction.

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

   * **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.

4. Prepare isopropanol.

Pretreatment of Samples

Plasma from whole blood

1. Centrifuge the blood samples at 2,000 x *g* for 10 minutes at 4°C.
2. Transfer the supernatant to a new 50 ml tube.
3. Repeat the step 1.

   *** Note:** The purpose of double spinning plasma is to remove all cellular contaminants and cell debris.
4. Transfer the supernatant to a new 50 ml tube.

Serum from whole blood

1. Allow the blood samples to clot for 30-60 minutes at room temperature before centrifugation.
2. Centrifuge the samples at 2,000 x *g* for 10 minutes.
3. Transfer the supernatant to a new 50 ml tube.

Urine and Saliva

1. Centrifuge the samples at 3,000 x *g* for 30 minutes at 4°C.
2. Transfer the supernatant to a new 50 ml tube.

cfDNA Extraction from Plasma/Serum/Urine/Saliva

1. Prepare the collected samples in page 11 (< 5 ml) to a 50 ml tube.

2. Add 40 µl of Proteinase K per 1 ml of sample and mix well by vortexing.

3. **(Lysis)** Add CL Buffer of the same volume as the sample volume to the sample and mix well by vortexing.
   *** Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

4. Incubate at 60°C for 10 minutes.

5. **(DNA precipitation)** Add 375 µl of isopropanol (not provided) per 1 ml of the lysate and mix well.

6. **(DNA binding)** Add 200 µl of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

7. Let stand for 5 minutes on ice or 10 minutes at room temperature.

8. Place the tube in *MagListo*™-50 Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

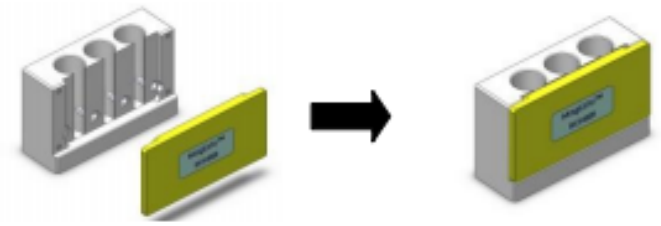


Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

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.

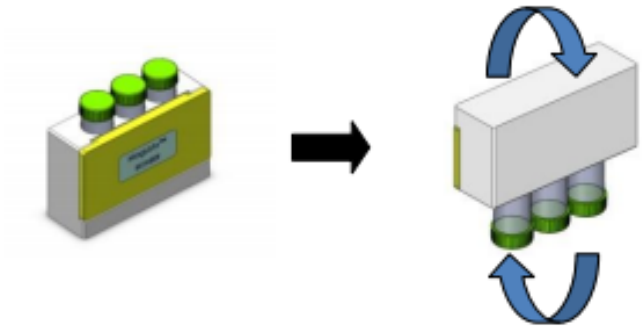


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

10. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 1 ml of CB Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

11. Take the CB Buffer mixed with magnetic nanobeads and transfer into the new 1.5 ml or 2 ml tube.

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

13. 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.

14. **(2nd Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 1 ml of CW1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended. Repeat steps 12-13 once more.

15. **(3rd Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 1 ml of TA Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended. Repeat steps 12-13 once more.

16. **(Elution)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 30 µl (mini)/ 50 µl (midi)/ 70 µl (maxi) of EA Buffer to each tube and resuspend DNA by pipetting or vortexing.

17. Incubate at 60°C for 1 minute and vortex the tubes for 15 seconds.

18. Attach the magnet plate to *MagListo*™ stand 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 the supernatant containing cfDNA carefully to a new tube.

Summary of Reagent Volumes Required in Each Step of DNA Extraction

Step	Buffer	Volume		
		Mini	Midi	Maxi
Sample	Plasma/Serum/Urine/Saliva	< 1 ml	< 3 ml	< 5 ml
Lysis	Proteinase K	40 µl	120 µl	200 µl
	CL Buffer	1 ml	3 ml	5 ml
DNA Precipitation	Isopropanol (not provided)	375 µl	1,125 µl	1,875 µl
DNA Binding	Magnetic Nano Bead	200 µl		
1st Washing	CB Buffer	1 ml		
2nd Washing	CW1 Buffer	1 ml		
3rd Washing	TA Buffer	1 ml		
Elution	EA Buffer	30 µl	50 µl	70 µl

Troubleshooting

1. No protein synthesis, including positive control protein

Problem	Comments
Low cfDNA yield	• **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.
	• **The lysis may have been incomplete.** It may take more time depending on the type of sample. For efficient lysis, you may perform shaking water bath or rocking platform.
	• **You may have used too much starting material.** Too much starting material causes incomplete lysis and neutralization. Appropriate amount of starting sample should be used for efficient extraction of cfDNA. For more information, refer to “Specifications” on page 2.
	• **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.
	• **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.
Low $A_{260/280}$ ratio	• **Magnetic Nano Beads may have been washed insufficiently.** You should always completely remove the remaining washing solution and dry beads completely in the drying

	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	• **CL Buffer, CB Buffer, and CW1 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	• **The DNA from old or incorrectly stored sample may often be degraded.** As the DNA yield is highly dependent on storage conditions of samples, please use fresh samples for optimal results. In case of using stored tissue sample, it is recommended to use sample stored at -70°C.
	• **Repeated freezing and thawing may degrade DNA.** Avoid repeated freezing and thawing.
Sample floating upon loading in an agarose gel	• **Sample may contain ethanol.** Floating is caused by remaining ethanol. Ensure that the drying 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™ cfDNA Extraction Kit	50 reactions	K-3619

Related Products

Description	Cat. No
Proteinase K Powder	KB-0111
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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