USER GUIDE

MagListo™ 5M Forensic Sample DNA Extraction Kit

Cat. No. K-3615

BIONEER
Innovation • Value • Discovery

MagListo™ 5M Forensic Sample DNA Extraction Kit

Kit for the extraction of genomic DNA from forensic sample

User Guide

K-3615

Σ 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 Forensic Sample DNA 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

This kit contains adequate reagents for 100 reactions.

Components	Amount	Storage
Proteinase K powder, lyophilized	25 mg x 2 ea	Refer to the “Storage” below.
Poly(A), lyophilized	1 mg x 1 ea	
Magnetic Nano Bead	1.8 ml x 6 ea	Store at room temperature (15-25°C).
TL Buffer (Lysis)	35 ml x 1 ea	
GB Buffer (Binding)	30 ml x 1 ea	
WM1 Buffer (1st Washing)	40 ml x 1 ea	
W2 Buffer (2nd Washing)	80 ml x 1 ea	
WE Buffer (3rd Washing)	80 ml x 1 ea	
EA Buffer (Elution)	15 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 (15-25°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.
To prepare poly(A) solution at a concentration of 1 µg/µl, add 1 ml of nuclease-free water to the lyophilized Poly(A). Store the solution in small aliquots at -20°C.

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

Specifications

***MagListo*™ 5M Forensic Sample DNA Extraction Kit**		
Sample Type	Various kinds of forensic sample	
Turnaround Time	< 10 min	
Typical DNA Yield	Up to 5 μg	
DNA Purity	A_{260}/A_{280} > 1.8	
Isolation Technology	Magnetic Nano Bead	
Amount of Starting Sample	Forensic Samples	Refer to the page 11.
	Tissue	< 10 mg
	Urine	< 15 ml
	Blood and Saliva	1-100 μl

*** 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 GB Buffer and WM1 Buffer contain chaotropic salts which are irritants.

Introduction

Product Description

MagListo™ 5M Forensic Sample DNA Extraction Kit is designed for extraction of highly purified total DNA from a variety of forensic sample, such as whole blood, saliva, dried body fluid spot, fingerprint, nail clipping, or hair. The kit employs Magnetic Nano Beads to extract total DNA 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). 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, SNP genotyping, and Short Tandem Repeat (STR) analysis.

Principle

MagListo™ 5M Forensic Sample DNA Extraction Kit is designed for extraction of total DNA from a variety of sources including high molecular weight DNA (up to 40 kb). 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. Total DNA extraction using silica-coated magnetic nanobeads.

Features & Benefits

- Comprehensive: High quality and yield of total DNA extraction from a broad range of forensic samples such as whole blood, saliva, urine, fingerprints, hair, nails, or bones.
- Rapid: Magnetic Nano Beads enable the rapid nucleic acid extraction.
- Cost-effective: Can be applied to *ExiPrep*™ 96 Lite to automate DNA 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*™-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 following are recommended when handling the *MagListo*™ Magnetic Separation Rack

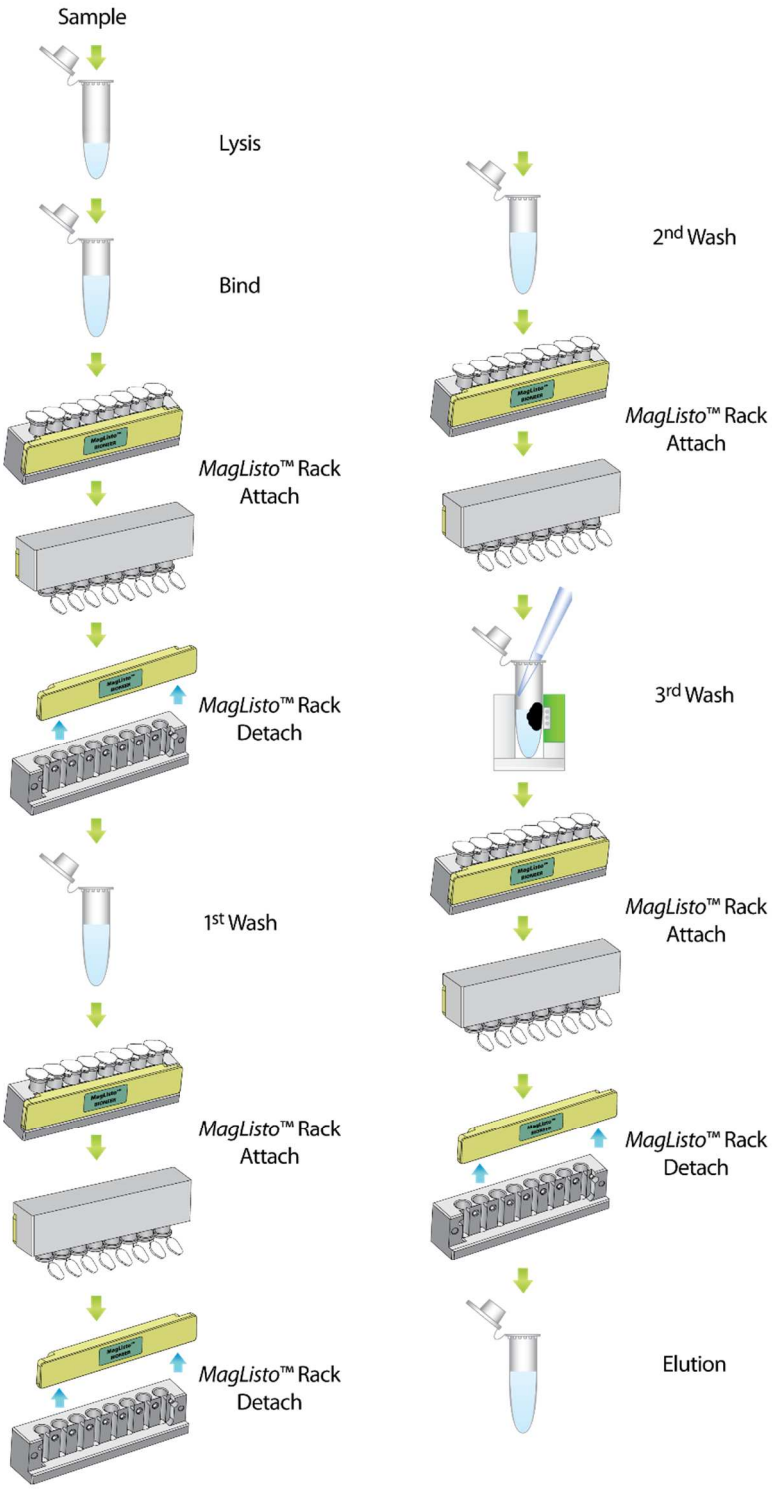
- The product is made of acryl and plastic. Be careful not to drop the product as the dropping may break the product.
- When moving the product, take extra care not to drop the product as it may cause injury.
- If the product is broken, do not discard it with bare hands as the sharp edges may cause injury.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

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. Pre-heat EA Buffer at 60°C before use. This process is essential for maximal recovery in filter-based extraction.

3. Completely dissolve Poly(A) in 1,000 µl of nuclease-free water before use. Store the solution in small aliquots at -20°C.

4. Add indicated volume of absolute ethanol (not provided) to WM1 Buffer before use (see bottle label).

5. Incubate the TL Buffer at 60°C when it has precipitates.

6. 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.

Pretreatment of Samples

Dried body fluid spot or fingerprint (FTA card, paper, cloth, etc.)

Punch out the sample up to 7 mm diameter with a single-hole paper puncher or cut out up to 2 cm^2. Cut the sample into smaller pieces to increase lysis efficiency.

Hair

Cut the hair 1 cm length from the hair root end. Cut the sample into smaller pieces to increase lysis efficiency.

Bone and teeth

Grind (Homogenize) the bone or teeth (up to 100 mg) to a fine powder.

Chewing gum

Cut the chewing gum (up to 30 mg) into smaller pieces to increase lysis efficiency.

Cigarette butts

Cut out up to 2 cm^2 from the end of the cigarette butt.

Buccal swab

Cut the swab from its stick by hand or scissors. Use single piece of swab for nucleic acid extraction.

Preparing Lysates from Forensic Sample

1. Apply the collected samples to 1.5 ml tube.

2. **(Lysis)** Add 300 µl of TL Buffer and 10 µl of Proteinase K to the sample and mix well by vortexing.

3. **(Optional)** Add 20 µl of 1 M DTT (not provided) to the sample and mix well by vortexing.
   * **Note:** If the sample is hair, nail clipping, or semen stains, this step is necessary to increase the DNA yield.

4. Incubate at 60°C for at least 1 hour.
   * **Note:** It may take more time depending on the type of sample or age of starting material. If the sample is hair, nail clipping, or semen stains, you should extend the incubation time up to overnight.

5. Add 300 µl of GB Buffer to the lysate and mix well by vortexing.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

6. **(Optional)** Add 2 µl of Poly(A) and mix well.

7. Incubate at 60°C for 20 minutes.

8. Centrifuge at 13,000 rpm for 1 minute.

9. Carefully apply the cleared supernatant to a clean 1.5 ml or 2 ml tube.

10. **(DNA precipitation)** Add 600 µl of absolute ethanol (not provided) to the lysate and mix well by vortexing. Briefly spin down to collect lysates clinging under the lid.

11. Proceed immediately to “Purification Procedure Using Magnetic Nano Bead” on page 16.

Preparing Lysates from Tissue

1. Grind (or homogenize) < 10 mg of tissue sample with a mortar and pestle (or homogenizer) and place them into a clean 1.5 ml or 2 ml tube. Hard tissue can be ground to a fine powder in liquid nitrogen†.

   * **Note:** If the sample is not ground completely, it will result in significantly reduced DNA yields. Final DNA yield depends on the amount and type of tissue.

   † After grinding, liquid nitrogen should be evaporated.

2. **(Lysis)** Add 90 µl of TL Buffer.

3. Add 10 µl of Proteinase K and mix well by vortexing.

4. **(Optional)** If RNA-free DNA is required, add up to 200 µg of RNase A (not provided, Cat. No. KB-0101) to the sample, gently mix, and incubate for 2 minutes at room temperature.

5. Incubate at 60°C until the sample has been completely lysis.

6. Add 100 µl of GB Buffer to the sample and mix well by vortexing.

   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

7. **(DNA precipitation)** Add 200 µl of absolute ethanol (not provided) to the lysate and mix well by vortexing. Briefly spin down to collect lysates clinging under the lid.

8. Proceed immediately to “Purification Procedure Using Magnetic Nano Bead” on page 16.

Preparing Lysates from Urine

1. Centrifuge the urine sample and discard the supernatant.
   1) (< 2 ml) Centrifuge at 8,000 rpm (6,000 x *g*) for 2 minutes.
   2) (< 15 ml) Centrifuge at 3,500 rpm (2,000 x *g*) for 10 minutes.

2. Resuspend the sample in 500 µl of PBS Buffer by vortexing or pipetting. Transfer the sample into a 1.5 ml or 2 ml tube.

3. Centrifuge at 8,000 rpm for 2 minutes and discard the supernatant.

4. **(Lysis)** Add 300 µl of TL Buffer and 10 µl of Proteinase K and mix well by vortexing.

5. **(Optional)** Add 10 µl of 1 M DTT (not provided) to the sample and mix well by vortexing.
   *** Note:** If the urine contains sperm cells, this step will improve the DNA yield.

6. Incubate at 60°C for 1 hour.

7. Add 300 µl of GB Buffer to the sample and mix well by vortexing.
   *** Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

8. Incubate at 60°C for 10 minutes.

9. **(DNA precipitation)** Add 600 µl of absolute ethanol (not provided) to the lysate and mix well by vortexing. Briefly spin down to collect lysates clinging under the lid.

10. Proceed immediately to “Purification Procedure Using Magnetic Nano Bead” on page 16.

Preparing Lysates from Blood and Saliva

1. Apply 1-100 µl of blood or saliva to a clean 1.5 ml or 2 ml tube.
   * **Note:** If the sample volume is less than 100 µl, adjust the total volume to 100 µl by adding PBS buffer (not provided).

2. Add 10 µl of Proteinase K to the sample.

3. **(Lysis)** Add 100 µl of GB Buffer 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 200 µl of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysates clinging under the lid.

6. Proceed immediately to “Purification Procedure Using Magnetic Nano Bead” on page 16.

Purification Procedure Using Magnetic Nano Bead

1. **(DNA binding)** Add 100 µl of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

2. 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.

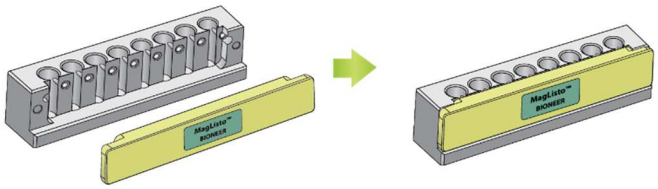


Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

3. 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.

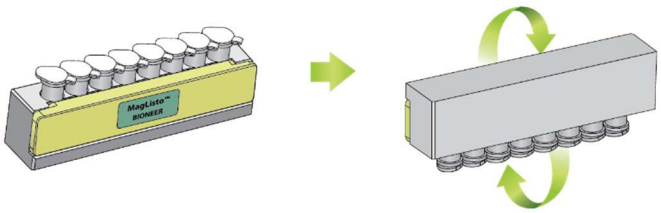


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

4. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 700 µl of WM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

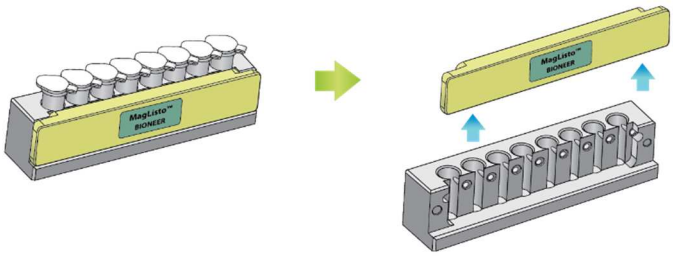


Figure 4. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

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

6. 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.

7. **(2nd Washing)** Repeat steps 4-6 by adding 700 µl of W2 Buffer for additional washing. Repeat steps 5-6 once more.

8. **(3rd Washing)** Without removing the tubes from *MagListo*™ Magnetic Separation Rack, add 700 µl 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.

9. Discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

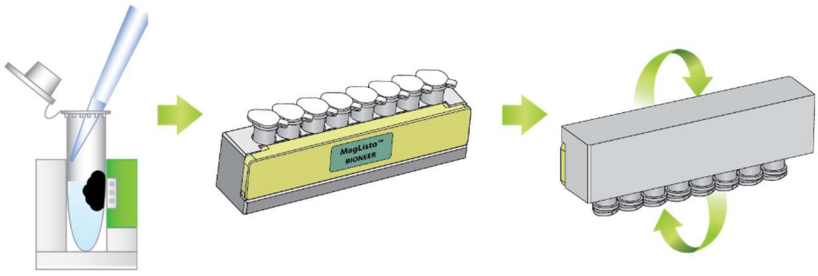


Figure 5. Washing the beads to remove residual ethanol. Please refer to the information above.

*** 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.

10. **(Elution)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 30-80 µl of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.

11. Incubate at 60°C for 1 minute and vortex for 15 seconds.

12. 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.

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

14. Discard the tubes with the remaining beads.

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

DNA Clean-Up

1. Transfer the eluted DNA or enzyme reaction products to a clean 1.5 ml or 2 ml tube.

2. **(Optional)** If RNA-free DNA is required, add up to 200 µg of RNase A (not provided, Cat. No. KB-0101) to the sample, gently mix, and incubate for 2 minutes at room temperature.

3. **(Binding)** Add 1 volume of GB Buffer to 1 volume of the eluted DNA and mix well by vortexing.

4. **(DNA precipitation)** Add 3 volumes of absolute ethanol (not provided) to 1 volume of the eluted DNA and mix well by vortexing.

5. **(DNA binding)** Add 100 µl of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

6. 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.

7. 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.

8. **(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.

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

10. 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.

11. Go to step 8 of “Purification Procedure Using Magnetic Nano Bead” on page 17.

Summary of Reagent Volumes Required in Each Step of DNA Extraction

DNA Extraction from Forensic Sample

Step	Buffer	Volume
	Forensic sample	
Lysis	TL Buffer	100-300 µl
	GB Buffer	100-300 µl
DNA Precipitation	Absolute ethanol	200-600 µl
DNA Binding	Magnetic Nano Bead	100 µl
1st Washing	WM1 Buffer	700 µl
2nd Washing	W2 Buffer	700 µl
3rd Washing	WE Buffer	700 µl
Elution	EA Buffer	30-80 µl

Troubleshooting

Problem	Comments
Low DNA 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, especially in the case of tissue sample.** 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 DNA. 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.** 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.
Presence of RNA in the eluted DNA	• **RNA may be present in the eluted DNA when both DNA and RNA are resent in the sample.** If RNA-free DNA is required, add RNase A (Cat. No. KB-0101) to the sample before adding GB Buffer. For more information, refer to “DNA Clean-Up” on page 19.
Aggregation of Magnetic Nano Beads	• **You may use 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	• **TL Buffer and GB 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 3rd washing step in the protocol is properly performed.

References

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 Forensic Sample DNA Extraction Kit	100 reactions	K-3615

Related Products

Description	Cat. No
Proteinase K Powder	KB-0111
RNase A Powder	KB-0101
Phosphate Buffered Saline (PBS)	C-9024
MagListo™-2 Magnetic Separation Rack	TM-1010

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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