USER GUIDE

MagListo™ 5M Genomic DNA Extraction Kit

Cat. No. K-3603

BIONEER
Innovation • Value • Discovery

MagListo™ 5M Genomic DNA Extraction Kit

Kit for the extraction of genomic DNA from whole blood, animal tissues, or cultured cells

User Guide

K-3603

Σ 100

Version No.: 5 (2023-06-22)

Please read all the information in booklet before using the unit

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Intended Use

MagListo™ 5M Genomic 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

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Copyright

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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.
RNase A powder, lyophilized	24 mg x 2 ea	
Magnetic Nano Bead	11 ml x 1 ea	Store at room temperature (15-25°C).
TL Buffer (Tissue Lysis)	35 ml x 1 ea	
GB Buffer (Binding)	30 ml x 1 ea	
WM1 Buffer (1st Washing)	60 ml x 1 ea	
W2 Buffer (2nd Washing)	80 ml x 1 ea	
WE Buffer (3rd Washing)	120 ml x 1 ea	
EA Buffer (Elution)	25 ml x 1 ea	
One Page Protocol	1 ea	

* Mini – 100 rxn, Midi – 15 rxn, Maxi – 7 rxn

Storage

The kit will maintain performance for at least three 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 and RNase A should be completely dissolved in 1,250 µl and 600 µl of nuclease-free water, respectively. For short term storage, dissolved Proteinase K and RNase A 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

	Amount of Starting Sample (Typical DNA Yield)		
Sample Type	**Mini**	**Midi**	**Maxi**
Whole blood	200 µl (< 10 µg)	2 ml (< 80 µg)	4 ml (< 150 µg)
Cultured cells†	< 1 x 10^6 cells (< 12 µg)	< 5 x 10^6 cells (< 60 µg)	< 1 x 10^7 cells (< 120 µg)
Animal tissues	< 25 mg (< 10 µg)	< 100 mg (< 40 µg)	< 250 mg (< 120 µg)
Bacterial cells (Gram (-), (+))	< 1 x 10^9 cells (< 15 µg)	< 5 x 10^9 cells (< 80 µg)	< 1 x 10^{10} cells (< 150 µg)
DNA purity	A_{260}/A_{280} > 1.8		

*** Note:** The DNA yield from samples with a low number of cells may be less than the figures shown in the table.

† For cultured cells, samples with cell number of < 1 x 10^4 cells can be used for “micro” scale extraction.

Extraction of genomic DNA from small amount of sample

MagListo™ 5M Genomic DNA Extraction Kit can extract genomic DNA from small amount of sample. If the sample contains low number of cells (< 1 x 10^4 cells) or has small amount of DNA, it is recommended to add about 4 µg of carrier RNA to the starting sample. Carrier RNA can be removed later by RNase digestion. Please see “DNA Extraction from Cultured Cells for Micro Scale” on page 15 for more details.

Recommended amounts of starting sample

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

Table 1. Growth area and average cell yield in various culture dishes.

Cell Culture Dishes	Growth Area (cm^2)	Average Cell Yield
Multi well plates		
6-well	9.6	1.2×10^6
12-well	4	4×10^5
24-well	2	2×10^5
48-well	1	1×10^5
96-well	0.35-0.6	4×10^4
Dishes		
35 mm	8	1.2×10^6
60 mm	21	3×10^6
100 mm	55	8×10^6
150 mm	148	2×10^7
Flasks		
50 ml	25	2.5×10^6
300 ml	75	1×10^7

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because GB Buffer and WM1 Buffer contain chaotropic salts which are irritants.
- Thoroughly mix TL Buffer and GB Buffer by shaking before use.

Introduction

Product Description

MagListo™ 5M Genomic DNA Extraction Kit is designed for extraction of highly purified total DNA from whole blood, animal tissues, cultured cells, and bacterial cells. The kit employs Magnetic Nano Beads to extract genomic 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 gene cloning, PCR, Real-time PCR, southern blotting, and SNP genotyping.

Principle

MagListo™ 5M Genomic DNA Extraction Kit is designed for extraction of genomic DNA 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 genomic 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 genomic DNA is eluted in an elution buffer or nuclease-free water.

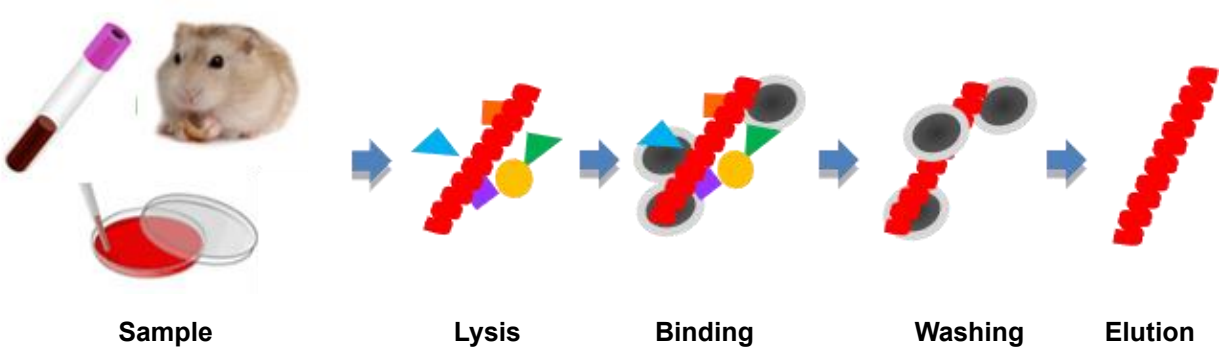


Figure 1. Genomic DNA extraction using silica-coated magnetic nanobeads.

Features & Benefits

- Comprehensive: High quality and yield of genomic DNA extraction from various samples such as whole blood, animal tissues, cultured cells, or bacterial cells.
- 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: A wide range of possible sample sizes even with a cell count less than 1 x 10^4 cells.
- 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 followings are recommended when handling the *MagListo*™ Magnetic Separation Rack.

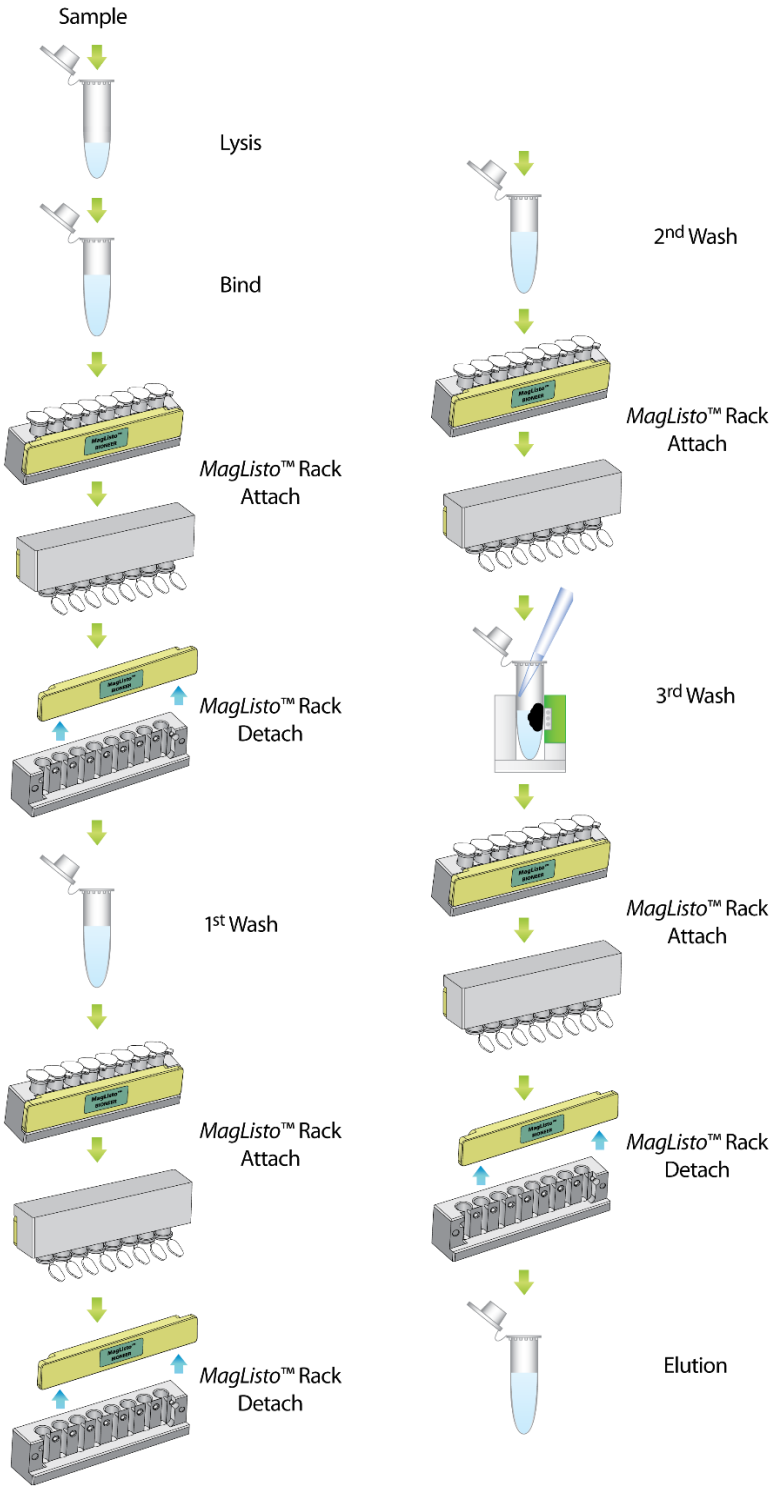
- The product is made of acryl and plastic. Be careful not to drop the product as the dropping may break the product.
- When moving the product, take extra care not to drop the product as it may cause injury.
- If the product is broken, do not discard it with bare hands as the sharp edges may cause injury.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

Blood

Blood sample should immediately be used or 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.

Cultured cells

Cultured cells can easily be harvested by centrifugation. However, it might be difficult to extract genomic DNA if cultured cells are too clustered. In this case, trypsin can be used to detach each cell from the cluster.

For genomic DNA extraction with a *MagListo*™ 5M Genomic DNA Extraction Kit, number of cells should be less than 1 x 10^7 cells, which is calculated with a cell counter. It is recommended to keep the samples on ice until use.

Tissue

Tissue samples should immediately be used or stored at -70°C upon harvest. To disrupt tissue sample, grind it with a mortar and pestle in liquid nitrogen. Alternatively, a homogenizer or a bead-beater can be used.

Bacterial cells

Bacterial cells can be processed in a shaking incubator for 12-16 hours at 37°C. Optimal results can be obtained when harvested bacterial cells are immediately used or stored at between -20°C and -80°C. Additional bacteriolytic agents like lysozyme or lysostaphin should be used to break the multilayered cell wall of gram-positive bacteria. For gram-negative bacteria, these agents are not needed.

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. Completely dissolve RNase A powder in 600 µl of nuclease-free water before use. Dissolved RNase A should be stored at 4°C.

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

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.

DNA Extraction from Whole Blood for Mini/Midi/Maxi Scale

1. Apply 200 µl (mini)/ 2 ml (midi)/ 4 ml (maxi) of whole blood or buffy coat sample to the indicated clean tube below.
   1) (Mini) Add Proteinase K to a 1.5 ml or 2 ml tube.
   2) (Midi) Add Proteinase K to a 15 ml tube.
   3) (Maxi) Add Proteinase K to a 50 ml tube.
   * **Note:** If the sample volume is less than the indicated volume above, adjust the total volume to 200 µl (mini)/ 2 ml (midi)/ 4 ml (maxi) by adding PBS buffer (not provided).

2. Add 20 µl (mini)/ 100 µl (midi)/ 200 µl (maxi) of Proteinase K to the sample.

3. **(Lysis)** Add 200 µl (mini)/ 2 ml (midi)/ 4 ml (maxi) 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 400 µl (mini)/ 4 ml (midi)/ 8 ml (maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysates clinging under the lid.

6. **(DNA binding)** Add 100 µl (mini)/ 500 µl (midi)/ 1 ml (maxi) of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

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

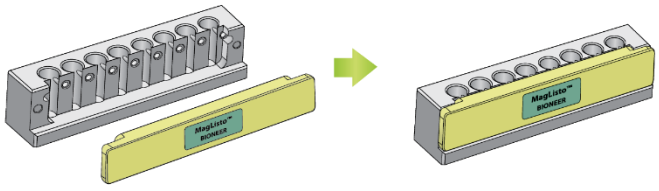


Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

8. 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.

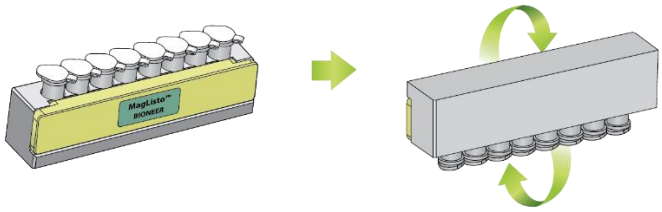


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

9. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 500 µl (mini)/ 3 ml (midi)/ 5 ml (maxi) of WM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

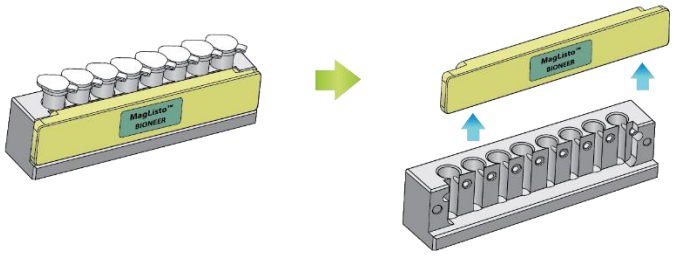


Figure 4. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

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

11. 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.

12. Repeat steps 9-11.

13. **(2nd Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack.

Repeat steps 9-11 by adding 700 µl (mini)/5 ml (midi)/10 ml (maxi) of W2 Buffer for additional washing. Repeat steps 10-11 once more.

14. **(3rd Washing)** Without removing the tubes from *MagListo*™ Magnetic Separation Rack, add 700 µl (mini)/ 8 ml (midi)/ 15 ml (maxi) 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.

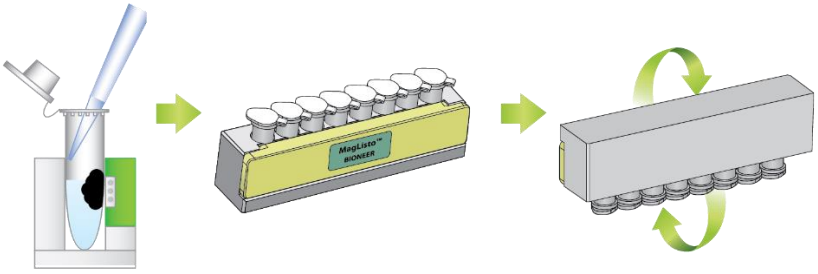


Figure 5. Washing the beads to remove residual ethanol. Please refer to the information above.

*** 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)/ 500 µl (midi)/ 1 ml (maxi) of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.

17. Incubate tubes 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.

DNA Extraction from Cultured Cells for Micro/Mini/Midi/Maxi Scale

1. Harvest cultured cells [< 1 x 10^4 cells (micro)/ < 1 x 10^6 cells (mini)/ < 5 x 10^6 cells (midi)/ < 1 x 10^7 cells (maxi)] by centrifugation at 300 x *g* for 10 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet.

2. Resuspend the cell pellet from step 1 in 100 µl (micro)/ 200 µl (mini)/ 1 ml (midi, maxi) of PBS buffer (not provided) and transfer the resuspended cells to the indicated clean tube below.
   1) (Micro/ Mini) Transfer the resuspended cells to a 1.5 ml or 2 ml tube.
   2) (Midi/ Maxi) Transfer the resuspended cells to a 15 ml tube.

3. Add 10 µl (micro)/ 20 µl (mini)/ 100 µl (midi)/ 200 µl (maxi) of Proteinase K to the sample.

4. If RNA-free genomic DNA is required, add up to 2 µl (micro)/ 10 µl (mini)/ 75 µl (midi)/ 150 µl (maxi) of RNase A to the sample, gently mix, and incubate for 2 minutes at room temperature.

5. **(Lysis)** Add 100 µl (micro)/ 200 µl (mini)/ 1 ml (midi, maxi) of GB Buffer to the sample and mix well by vortexing.
   *** Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

6. Incubate at 60°C for 10 minutes.

7. **(DNA precipitation)** Add 200 µl (micro)/ 400 µl (mini)/ 2 ml (midi, maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysates clinging under the lid.

8. **(DNA binding)** Add 100 µl (micro, mini)/ 500 µl (midi)/ 1 ml (maxi) of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

9. Place the tube in *MagListo*™-2 (micro, mini)/ *MagListo*™-15 (midi, maxi) Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

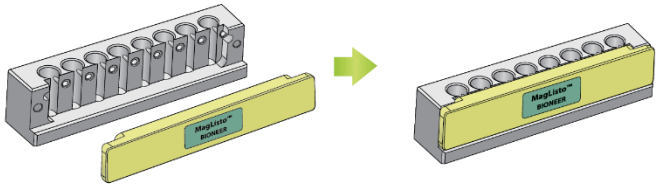


Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

10. 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.

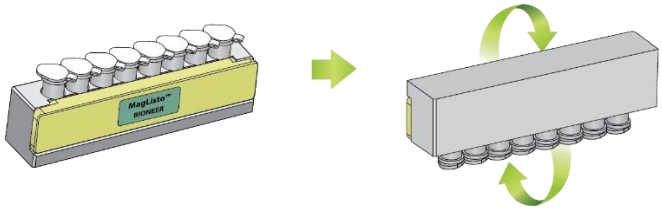


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

11. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 700 µl (micro, mini)/ 5 ml (midi)/ 10 ml (maxi) of WM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

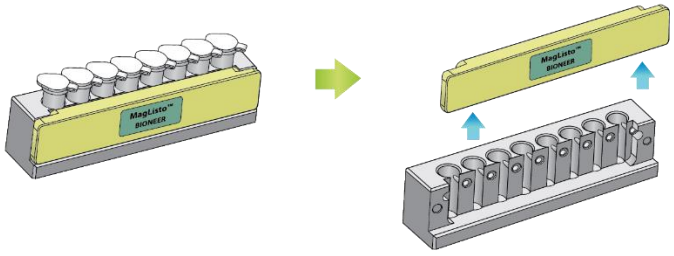


Figure 4. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

12. Attach the magnet plate to *MagListo*™ stand 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)** Repeat steps 11-13 by adding 700 µl (micro, mini)/ 5 ml (midi)/ 10 ml (maxi) of W2 Buffer for additional washing. Repeat steps 12-13 once more.

15. **(3rd Washing)** Without removing the tubes from *MagListo*™ Magnetic Separation Rack, add 700 µl (micro, mini)/ 8 ml (midi)/ 15 ml (maxi) 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.

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

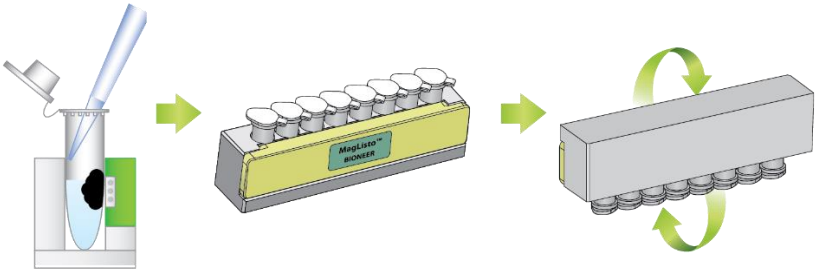


Figure 5. Washing the beads to remove residual ethanol. Please refer to the information above.

*** 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.

17. **(Elution)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 50 µl (micro)/ 100 µl (mini)/ 500 µl (midi)/ 1 ml (maxi) of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.

18. Incubate at 60°C for 1 minute.

19. 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.

20. Without removing the tube from *MagListo*™ Magnetic Separation Rack, transfer

supernatant containing DNA carefully to a new tube.

21. Discard the tubes with the remaining beads.

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

DNA Extraction from Animal Tissue for Mini/Midi/Maxi Scale

1. **(Homogenization)** Grind (or homogenize) < 25 mg (mini)/ < 100 mg (midi)/ < 250 mg (maxi) of fresh or thawed animal tissue sample with a mortar and pestle (or homogenizer) and place them into the indicated clean tube below. Hard tissue can be ground to a fine powder in liquid nitrogen†.
   1) (Mini) Transfer the homogenized tissue sample to a 1.5 ml or 2 ml tube.
   2) (Midi) Transfer the homogenized tissue sample to a 15 ml tube.
   3) (Maxi) Transfer the homogenized tissue sample to a 50 ml tube.

   *** Note:** If the sample is not ground completely, it will result in significantly reduced DNA yields.

   † After grinding, liquid nitrogen should be evaporated.

2. **(Lysis)** Add 180 µl (mini)/ 1.8 ml (midi)/ 3.6 ml (maxi) of TL Buffer.

3. Add 20 µl (mini)/ 100 µl (midi)/ 200 µl (maxi) of Proteinase K and mix well by vortexing.

4. If RNA-free genomic DNA is required, add up to 10 µl (mini)/ 75 µl (midi)/ 150 µl (maxi) of RNase A 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 200 µl(mini)/ 2 ml (midi)/ 4 ml (maxi) 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 400 µl (mini)/ 4 ml (midi)/ 8 ml (maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysates clinging under the lid.

8. **(DNA binding)** Add 100 µl (mini)/ 500 µl (midi)/ 1 ml (maxi) of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

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

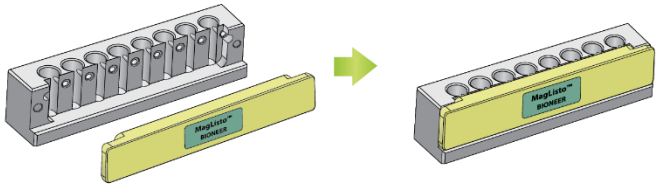


Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

10. 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.

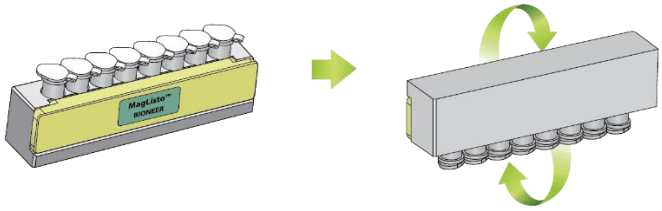


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

11. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 700 µl (mini)/ 5 ml (midi)/ 10 ml (maxi) of WM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

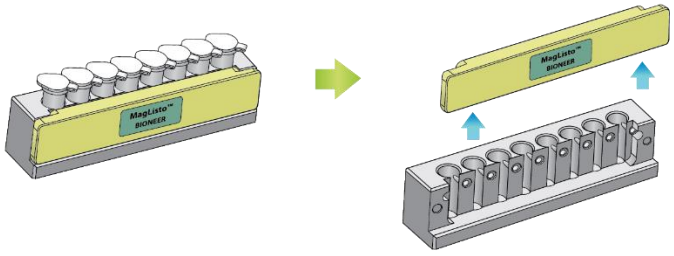


Figure 4. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

12. Attach the magnet plate to *MagListo*™ stand 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)** Repeat steps 11-13 by adding 700 µl (mini)/ 5 ml (midi)/ 10 ml (maxi) of W2 Buffer for additional washing. Repeat steps 12-13 once more.

15. **(3rd Washing)** Without removing the tubes from *MagListo*™ Magnetic Separation Rack, add 700 µl (mini)/ 8 ml (midi)/ 15 ml (maxi) 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.

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

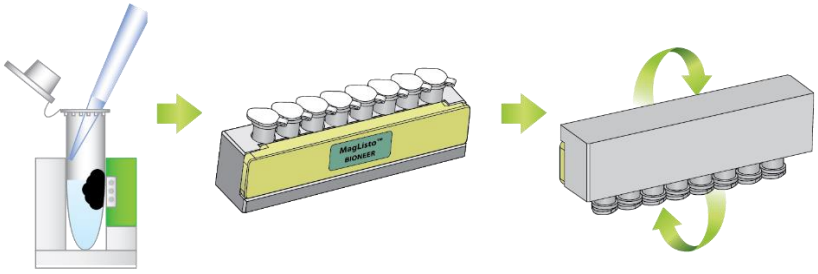


Figure 5. Washing the beads to remove residual ethanol. Please refer to the information above.

*** 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.

17. **(Elution)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 100 µl (mini)/ 500 µl (midi)/ 1 ml (maxi) of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.

18. Incubate at 60°C for 1 minute.

19. 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.

20. Without removing the tube from *MagListo*™ Magnetic Separation Rack, transfer

supernatant containing DNA carefully to a new tube.

21. Discard the tubes with the remaining beads.

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

DNA Extraction from Bacterial Cells (Gram-Negative Bacteria) for Mini/Midi/Maxi Scale

1. Harvest up to 1 x 10^9 (mini)/ 5 x 10^9 (midi)/ 1 x 10^{10} (maxi) bacterial cells by centrifugation at 6000 x *g* for 10 minutes to pellet cells. Discard the supernatant carefully with a pipette.

2. Resuspend the cell pellet from step 1 in 180 µl (mini)/ 1.8 ml (midi)/ 3.6 ml (maxi) of TL Buffer by vortexing or pipetting. Transfer the cell suspension into the indicated clean tube below.
   1) (Mini) Transfer the homogenized tissue sample to a 1.5 ml or 2 ml tube.
   2) (Midi) Transfer the homogenized tissue sample to a 15 ml tube.
   3) (Maxi) Transfer the homogenized tissue sample to a 50 ml tube.

3. Go to step 3 of “DNA Extraction from Animal Tissue for Mini/Midi/Maxi Scale” on page 19.

DNA Extraction from Bacterial Cells (Gram-Positive Bacteria) for Mini/Midi/Maxi Scale

1. Harvest up to 1 x 10^9 (mini)/ 5 x 10^9 (midi)/1 x 10^{10} (maxi) bacterial cells by centrifugation at 6000 x *g* for 10 minutes to pellet cells. Discard the supernatant carefully with a pipette.

2. Resuspend the cell pellet from step 1 in 180 µl (mini)/ 1.8 ml (midi)/ 3.6 ml (maxi) of lysis buffer (for gram-positive bacteria, not provided) by vortexing or pipetting. Transfer the cell suspension into the indicated clean tube below.
   1) (Mini) Transfer the homogenized tissue sample to a 1.5 ml or 2 ml tube.
   2) (Midi) Transfer the homogenized tissue sample to a 15 ml tube.
   3) (Maxi) Transfer the homogenized tissue sample to a 1.5 ml or 2 ml tube.

   *** Note:** Lysis buffer for gram-positive bacteria can be prepared by using this formulation: 20 mM Tris-HCl (pH 8.0), 2 mM sodium EDTA, and 1.2% Triton® X-100.

3. Add 20 µl (mini)/ 100 µl (midi)/ 200 µl (maxi) of lysozyme (100 mg/ml, not provided) and mix well by vortexing.

4. If RNA-free genomic DNA is required, add up to 10 µl (mini)/ 75 µl (midi)/ 150 µl (maxi) of RNase A to the sample and gently mix.

5. Incubate at 37°C for 30 minutes.

6. Add 20 µl (mini)/ 100 µl (midi)/ 200 µl (maxi) of Proteinase K and mix well by vortexing.

7. Add 200 µl (mini)/ 2 ml (midi)/ 4 ml (maxi) of GB Buffer and mix well by vortexing.

8. Incubate at 60°C for 30 minutes or until bacterial cells are completely lysed.

9. Go to step 7 of “DNA Extraction from Animal Tissue for Mini/Midi/Maxi Scale” on page 19.

DNA Clean-Up

1. Transfer the eluted DNA or enzyme reaction products into the indicated clean tube below.
   1) (Mini) Transfer the eluate to a 1.5 ml or 2 ml tube.
   2) (Midi/Maxi) Transfer the eluate to a 15 ml tube.

2. If RNA-free genomic DNA is required, add up to 10 µl (mini)/ 75 µl (midi)/ 150 µl (maxi) of RNase A 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 (mini)/ 500 µl (midi)/ 1 ml (maxi) 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 (mini)/*MagListo*™-15 (midi, maxi) Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

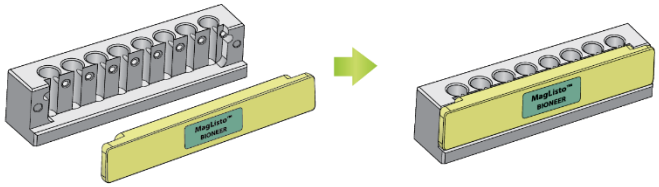


Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

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.

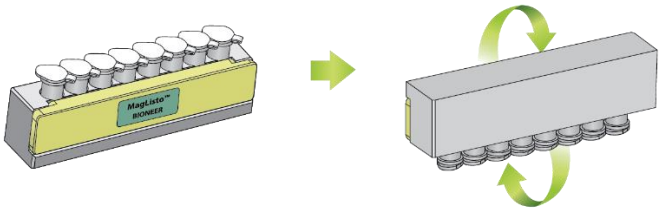


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

8. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 700 µl (mini)/ 5 ml (midi)/ 10 ml (maxi) of W2 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

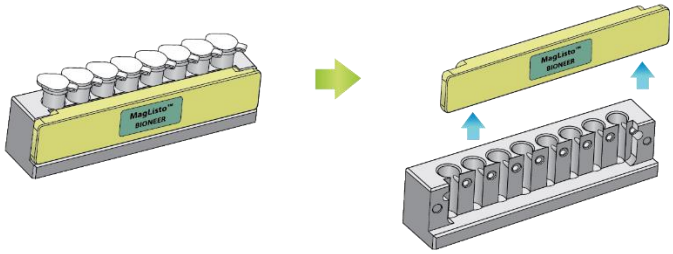


Figure 4. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

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 15 of “DNA Extraction from Animal Tissue for Mini/Midi/Maxi Scale” on page 21.

Summary of Reagent Volumes Required in Each Step of DNA Extraction

DNA Extraction from Whole Blood

Step	Buffer	Mini	Midi	Maxi
	Blood (+ PBS)	200 µl	2 ml	4 ml
Lysis	GB Buffer	200 µl	2 ml	4 ml
DNA Precipitation	Absolute ethanol	400 µl	4 ml	8 ml
DNA Binding	Magnetic Nano Bead	100 µl	500 µl	1 ml
1st Washing	WM1 Buffer	500 µl	3 ml	5 ml
2nd Washing	W2 Buffer	700 µl	5 ml	10 ml
3rd Washing	WE Buffer	700 µl	8 ml	15 ml
Elution	EA Buffer	100 µl	500 µl	1 ml

DNA Extraction from Cultured Cells

Step	Buffer	Micro	Mini	Midi	Maxi
	Culture Cells	< 1 x 10^4	< 1 x 10^6	< 5 x 10^6	< 1 x 10^7
Lysis	GB Buffer	100 µl	200 µl	1 ml	1 ml
DNA Precipitation	Absolute ethanol	200 µl	400 µl	2 ml	2 ml
DNA Binding	Magnetic Nano Bead	100 µl	100 µl	500 µl	1 ml
1st Washing	WM1 Buffer	700 µl	700 µl	5 ml	10 ml
2nd Washing	W2 Buffer	700 µl	700 µl	5 ml	10 ml
3rd Washing	WE Buffer	700 µl	700 µl	8 ml	15 ml
Elution	EA Buffer	50 µl	100 µl	500 µl	1 ml

DNA Extraction from Animal Tissue

Step	Buffer	Mini	Midi	Maxi
	Animal Tissue	< 25 mg	< 100 mg	< 250 mg
Lysis	TL Buffer	180 µl	1.8 ml	3.6 ml
	GB Buffer	200 µl	2 ml	4 ml
DNA Precipitation	Absolute ethanol	400 µl	4 ml	8 ml
DNA Binding	Magnetic Nano Bead	100 µl	500 µl	1 ml
1st Washing	WM1 Buffer	700 µl	5 ml	10 ml
2nd Washing	W2 Buffer	700 µl	5 ml	10 ml
3rd Washing	WE Buffer	700 µl	8 ml	15 ml
Elution	EA Buffer	100 µl	500 µl	1 ml

DNA Extraction from Bacterial Cells (Gram-Negative Bacteria)

Step	Buffer	Mini	Midi	Maxi
	Bacterial cells	< 1 x 10^9	< 5 x 10^9	< 1 x 10^{10}
Lysis	TL Buffer	180 µl	1.8 ml	3.6 ml
	GB Buffer	200 µl	2 ml	4 ml
DNA Precipitation	Absolute ethanol	400 µl	4 ml	8 ml
DNA Binding	Magnetic Nano Bead	100 µl	500 µl	1 ml
1st Washing	WM1 Buffer	700 µl	5 ml	10 ml
2nd Washing	W2 Buffer	700 µl	5 ml	10 ml
3rd Washing	WE Buffer	700 µl	8 ml	15 ml
Elution	EA Buffer	100 µl	500 µl	1 ml

DNA Extraction from Bacterial Cells (Gram-Positive Bacteria)

Step	Buffer	Mini	Midi	Maxi
	Bacterial cells	< 1 x 10^9	< 5 x 10^9	< 1 x 10^{10}
Lysis	Lysis Buffer (not provided)	180 µl	1.8 ml	3.6 ml
	Lysozyme (not provided)	20 µl	100 µl	200 µl
	GB Buffer	200 µl	2 ml	4 ml
DNA Precipitation	Absolute ethanol	400 µl	4 ml	8 ml
DNA Binding	Magnetic Nano Bead	100 µl	500 µl	1 ml
1st Washing	WM1 Buffer	700 µl	5 ml	10 ml
2nd Washing	W2 Buffer	700 µl	5 ml	10 ml
3rd Washing	WE Buffer	700 µl	8 ml	15 ml
Elution	EA Buffer	100 µl	500 µl	1 ml

Troubleshooting

Problem	Comments
Low genomic 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.** Ensure that sample changes clarity from turbid to clear for occurring protein digestion. Extend the incubation time if tissue sample is still not lysed. It may take more time depending on the type of tissue. If a cell mass still remains after the overnight incubation, centrifuge the sample and use supernatant for DNA extraction. 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 genomic 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.
	• **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.
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 genomic DNA is required, add RNase A to the sample before adding GB Buffer. For more information, refer to “DNA Clean-Up” on page 25.
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	• **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 (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 Genomic DNA Extraction Kit	100 reactions	K-3603

Related Products

Description	Cat. No
Proteinase K Powder	KB-0111
RNase A Powder	KB-0101
Phosphate Buffered Saline (PBS)	C-9024
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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