

User's Guide

***MagListo*[™]** **5M Plant Genomic DNA Extraction Kit**

REF

K-3604

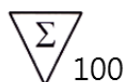
K-3605

MagListo™ 5M Plant Genomic DNA Extraction Kit

Kit for the extraction of genomic DNA from plant using *MagListo™*

User's Guide

K-3604 K-3605



Version No.: 1.0 (2015-04)

Please read all the information in booklet before using the unit



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I. Overview

Description

MagListo™ 5M Plant Genomic DNA Extraction Kit is an innovative product to extract total DNA from plant tissue of leaf and seed using magnetic nano beads along with *MagListo™* Magnetic Separation Rack. The use of *MagListo™* Magnetic Separation Rack greatly increases user convenience and saves process time without the need of centrifuge.

Features and Benefits

- Magnetic nano beads enable rapid extraction
- No need of costly instruments except *MagListo™* Magnetic Separation Rack

Applications

Gene Cloning, PCR, Real-Time PCR, Southern Blotting, SNP genotyping

II. Kit Components

<i>MagListo™</i> 5M Plant Genomic DNA Extraction Kit Cat. no. 3604, 3605	*K-3604 (8 reaction)	**K-3605 (100 reaction)
Buffer ① (Lysis)	4 ml x 1 ea	40 ml x 1 ea
Buffer ② (Precipitation)	1 ml x 1 ea	15 ml x 1 ea
Buffer ③ (1 st Washing)	6 ml x 1 ea	65 ml x 1 ea
Buffer ④ (2 nd Washing)	1.6 ml x 1 ea	16 ml x 1 ea
Buffer ⑤ (Elution)	1 ml x 1 ea	15 ml x 1 ea
Magnetic Nano Bead – DNA	1 ml x 1 ea	1.8 ml x 6 ea
Proteinase K	5 mg x 1 ea	25 mg x 2 ea
RNase A	6 mg x 1 ea	24 mg x 2 ea

*Mini – 8rxn, Midi – 1rxn **Mini – 100 rxn, Midi – 15 rxn, Maxi – 8 rxn

III. Storage

MagListo[™] 5M Plant Genomic DNA Extraction Kit should be stored dry at room temperature. It can be stored for up to 2 years if it remains sealed. *MagListo*[™] 5M Plant Genomic DNA Extraction Kit provides optimized Buffer ③ (1st Washing) which is poisonous and hazardous. Please, wear gloves and goggles eye protection when working with Buffer ③ (1st Washing).

IV. Intended Use

MagListo[™] 5M Plant Genomic DNA Extraction Kit is intended for research use only. This kit is not intended for human or veterinary diagnostics.

V. Safety Warnings and Precautions

Please inquire BIONEER's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

Before, during and after use of this kit as described in this User's Guide, all potentially hazardous materials (i.e. materials that may have come in contact with genetically recombinant samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/government in which this product is being used. A user must also be equipped with basic experimental techniques required for correct execution of the experiments described in this User's Guide.

Some applications that may be performed with this kit may infringe upon existing patents in certain countries. The purchase of this kit does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on country and application. We do not condone nor recommend the unlicensed use of a patented application.

VI. Warranty and Liability

All BIONEER products are manufactured and tested under strict quality control protocols. BIONEER guarantees the quality of all directly manufactured products during the warranty period of one (1) year from the date of purchase. If any issues are discovered relating to

compromise in product quality, immediately contact BIONEER's Customer Service Center (order@bioneer.com).

BIONEER does not assume liability for misuse of the product, i.e. usage of the product for any purposes other than its intended purpose as described in the User's Guide. BIONEER assumes liability under the condition that users disclose all information related to the problem to BIONEER in written form within 30 days of occurrence.

VII. Technical Assistance

At Bioneer, we pride ourselves on being responsive to your needs. If you have any questions or would like to find out more information about *MagListo*[™] products, please contact us. We look forward to hearing from you!

Technical Support

For all technical questions and troubleshooting on Bioneer products and applications.

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VIII. Quality Management

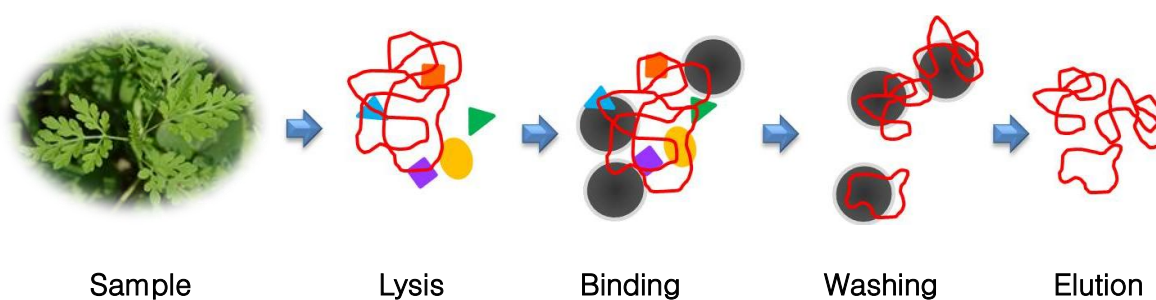
Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards. Each lot of *MagListo*[™] 5M Plant Genomic DNA Extraction Kit is carefully tested by the quality control team.

IX. Product Specifications

	Mini scale	Midi scale	Maxi scale
Tissue	~ 100 mg	~ 500 mg	~ 1 g
Seed	~ 50mg	~ 250mg	~ 500mg
Elution volume	100 ul	500 ul	1 ml
Typical DNA yield	5 ~ 15 ug	10 ~ 80 ug	30 ~ 150 ug
Expected purity	$A_{260/280} > 1.7$, $A_{260/230} > 1.4$		

X. Principle

The *MagListo*™ 5M Plant Genomic DNA Extraction Kit is designed for the extraction of total DNA from plant leaf and seed. The overall principle is based on adsorption of DNA onto the magnetic nano bead by chaotrophic salt. Guanidine hydrochloride as chaotropic agents in Buffer ③ (1st Washing), removes water molecules around DNA and silica coated magnetic bead surface resulting in DNA then being captured by silica coated 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 Buffer ⑤ (Elution), an aqueous solution with optimal pH.



XI. Materials and Equipment Needed But Not Provided

1. 1.5 ml tube, 2 ml tube, 15 ml tube
2. Vortex mixer
3. Table-top microcentrifuge, 16,000 x g (>13,000 rpm) (mini scale)
4. Centrifuge with rotor capable of 3,000 x g (midi & maxi scale)
5. Absolute ethanol
6. Thermal block or dry oven
7. Blow dryer or heat gun
8. Mortar and pestle
9. Ice
10. *MagListo*™ Magnetic Separation Rack

Magnetic Separation Rack Choice

Tube	<i>MagListo</i> ™ Magnetic Separation Rack
1 ml microcentrifuge tube	<i>MagListo</i> ™-8Ch Magnetic Separation Rack
1.5 ml or 2 ml microcentrifuge tube	<i>MagListo</i> ™-2 Magnetic Separation Rack
15 ml tube	<i>MagListo</i> ™-15 Magnetic Separation Rack
50 ml tube	<i>MagListo</i> ™-50 Magnetic Separation Rack

(Note) Please refer to the ordering information table on the latter part of the manual which contains the appropriate catalog number for specific size of tubes.

XII. Protocols

Before you begin

1. Completely dissolve Proteinase K in 250 μ l (KB-3604-7) or 1,250 μ l (KB-0111) of nuclease-free water.
2. Completely dissolve RNase A in 150 μ l (KB-0101) or 600 μ l (KB-3101) of nuclease-free water.
3. Buffer ③ (1st Washing) contains chaotropic salt. You should take the appropriate laboratory safety precautions and wear gloves when handling.
4. Add the correct amount of absolute ethanol to Buffer ③ (1st Washing) and Buffer ④ (2nd Washing).

a. DNA Extraction from Plant for Mini/Midi/Maxi

1. **(Homogenization)** Disrupt (or homogenize) the tissue ($\sim$ 100 mg (mini)/ $\sim$ 500 mg (midi)/ $\sim$ 1 g (maxi)) or seed ($\sim$ 50 mg (mini)/ $\sim$ 250 mg (midi)/ $\sim$ 500 mg (maxi)) and place them to each specific tube format.
 - a. **(Mini)** Place the homogenized tissue to a 1.5 ml or 2 ml tube
 - b. **(Midi/Maxi)** Place the homogenized tissue to a 15 ml tube
2. **(Lysis: 2-4)** Add 300 μ l (mini)/ 1.5 ml (midi)/ 3 ml (maxi) of Buffer ① (Lysis) and 10 μ l (mini)/ 75 μ l (midi)/ 150 μ l (maxi) of RNase A solution (see “Before you begin”) to the tube.
3. Add 20 μ l (mini)/ 100 μ l (midi)/ 200 μ l (maxi) of Proteinase K solution (see “Before you begin”) and mix well by vortex mixer. You must completely resuspend the sample to achieve maximum lysis efficiency.
4. Incubate at 60°C for 10min.
5. **(Precipitation: 5-6)** Add 100 μ l (mini)/ 500 μ l (midi)/ 1 ml (maxi) of Buffer ② (Precipitation) to the tube and mix well by vortex mixer. This step precipitates detergent, proteins and polysaccharides.
6. Incubate for 5 min (mini)/ 10 min (midi, maxi) on ice.

7. (Precipitates removal: 7–8) Centrifuge the tube at 16000 x g (>13,000 rpm) for 5 min (mini)/ 3,000 x g (> 4,000 rpm) for 15 min (midi, maxi) to obtain clear lysate.
8. Take the clear supernatant only and transfer into the new 1.5ml or 2ml tube (mini)/ 15 ml tube (midi, maxi).
9. (DNA Precipitation) Add 2 volumes of Buffer ③ (1st Washing) to the cleared lysate and mix well by vortex mixer.
10. (DNA binding with Magnetic Nano Bead: 10–12) Add 100 µl (mini)/ 500 µl (midi)/ 1 ml (maxi) of Magnetic Nano Bead Solution to the tube and mix well by vortex mixer.
(Note) Magnetic Nano Bead Solution contains magnetic nano beads. Please shake well before use.
11. Place the tube in MagListo-2 (mini)/ MagListo-15 (midi, maxi) stand and attach the magnet plate attached and invert the tube 3~4 times gently until the beads tightly bind to magnet.

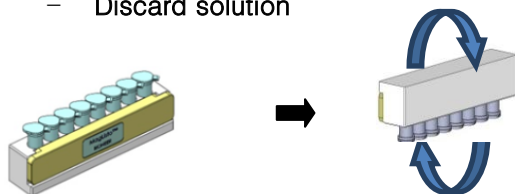
– Attachment



Combine the magnet plate to the stand.

12. Without removing the tube from *MagListo*™ rack, carefully pour the supernatant out and completely remove the remaining supernatant using paper towel by blotting.

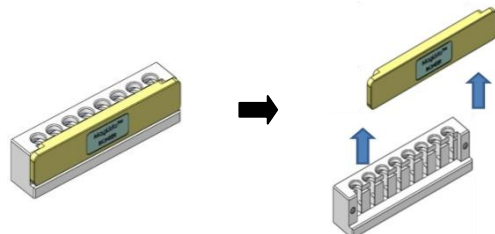
– Discard solution



Discard solution by inverting the *MagListo*™ rack. The **silicone immobilizer** inside the stand holds the tubes from falling in an upside down position. When discard solution, invert the rack completely for the solution not to smear on the rack.

13. (1st washing: 13–15) Detach the magnet plate from *MagListo*™ rack. Add 500 µl (mini)/ 3 ml (midi)/ 5 ml (maxi) of Buffer ③ (1st Washing) to the tube. Close the cap and mix by vortex mixer until the beads are fully resuspended.

– Detachment



Push up the magnet plate gently.

14. Attach the magnet plate to *MagListo*™ rack and invert the tube 3~4 times gently until the beads tightly bind to magnet.
15. Without removing the tube from *MagListo*™ rack, pour the supernatant out and completely remove the remaining supernatant using paper towel by blotting.
16. (2nd washing) Repeat the above step 13 ~ 15 by adding 700 µl (mini)/ 5 ml (midi)/ 8 ml (maxi) of Buffer ④ (2nd Washing) additional washing.
17. (3rd washing) Repeat the above step 13 ~ 15 by adding 700 µl (mini)/ 5 ml (midi)/ 8 ml (maxi) of absolute ethanol for additional washing.
18. (Drying) Completely dry the beads with the tube open and use a heat gun or a blowdryer for 1 min (mini)/ 3 min (midi)/ 5 min (maxi) 3 cm away from the top of the tube.
(Note) Without using heat gun or a blowdryer, place the device lying down in a dry oven at 60°C for 10 min (mini)/ 20 min (midi)/ 30 min (maxi).
19. (Elution: 19–23) Add 100 µl (mini)/ 500 µl (midi)/ 1 ml (maxi) of Buffer ⑤ (Elution) or distilled water to the tube with the magnet plate detached and resuspend completely by pipetting or vortex mixer for 15 sec.
20. Incubate the tube at 60°C for 1 min.

21. Attach the magnet plate to *MagListo*™ rack and invert the tube 3~4 times gently until the beads tightly bind to magnet.
22. Without removing the tube from *MagListo*™ rack, carefully take the supernatant containing DNA to a sterile microcentrifuge tube.
23. Discard the used magnetic nano beads. Do not reuse the beads.

Summary of reagents volume in each step of DNA Extraction from Plant

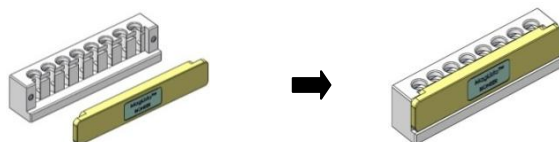
Step	Buffer	Mini	Midi	Maxi	Page
Sample	Plant Tissue	~ 100 mg	~ 500 mg	~ 1 g	P. 6
	Plant Seed	~ 50 mg	~ 250 mg	~ 500 mg	P. 6
Lysis	Buffer ① (Lysis)	300 µl	1.5 ml	3 ml	P. 6
Precipitation	Buffer ② (Precipitation)	100 µl	500 ul	1 ml	P. 6
DNA Precipitation	Buffer ③ (1 st Washing)	2 volumes of lysate			P. 6
Bead Binding	Magnetic Nano Bead	100 µl	500 µl	1 ml	P. 6
1 st Washing	Buffer ③ (1 st Washing)	500µl	3 ml	5 ml	P. 7
2 nd Washing	Buffer ④ (2 nd Washing)	700µl	5 ml	8 ml	P. 8
3 rd Washing	Absolute Ethanol	700µl	5 ml	8 ml	P. 8
Elution	Buffer ⑤ (Elution)	100 µl	500 µl	1 ml	P. 8

b. DNA Clean-Up

1. Transfer the eluate or enzyme reaction product to each specific tube format.
 - a. (Mini) Transfer the eluate to a 1.5 ml or 2 ml tube
 - b. (Midi/Maxi) Transfer the eluate to a 15 ml tube
2. (Optional) If RNA-free genomic DNA is required, add up to 10 µl (mini)/ 75 µl (midi)/ 150 µl (maxi) of RNase A solution (see “Before you begin”) and incubate for 2 min at room temperature.
3. (Binding) Add 2 volumes of Buffer ③ (1st Washing) to the eluate and mix completely by vortex mixer.
4. (DNA precipitation) Add 2 volumes of absolute ethanol to the eluate and mix well by vortex mixer.
5. (DNA binding with Magnetic Nano Bead: 5–7) Add 100 µl (mini)/ 500 µl (midi)/ 1 ml (maxi) of Magnetic Nano Bead solution to the tube and mix thoroughly using a vortex mixer until the beads are fully resuspended.

(Note) Magnetic Nano Bead Solution contains magnetic nano beads. Please shake well before use.
6. Place the tube in MagListo™-2 (mini)/ MagListo™-15 (midi, maxi) stand and attach the magnet plate. Invert the rack with the tube 3~4 times gently until the beads tightly bind to magnet.

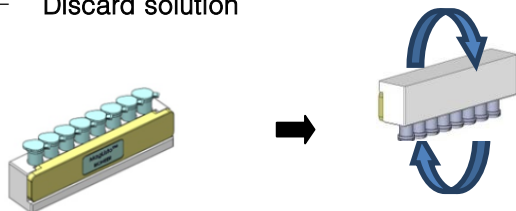
– Attachment



Combine the magnet plate to the stand.

7. Without removing the tube from *MagListo*™ rack, carefully pour the supernatant out and completely remove the remaining supernatant using paper towel by blotting.

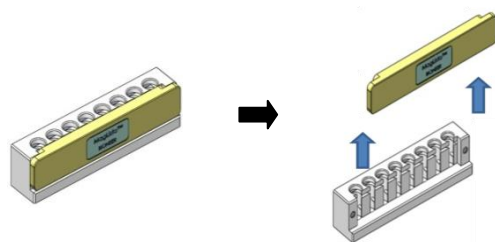
– Discard solution



Discard solution by inverting the *MagListo*™ rack. **The silicone immobilizer** inside the stand holds the tubes from falling in an upside down position. When discard solution, invert the rack completely for the solution not to smear on the rack.

8. (1st washing: 8–10) Detach the magnet plate from *MagListo*™ stand. Add 700 µl (mini)/ 5 ml (midi)/ 8 ml (maxi) of Buffer ④ (2nd Washing) to the tube. Close the cap and mix by vortex mixer until beads are fully resuspended.

– Detachment



Push up the magnet plate gently.

9. Attach the magnet plate to *MagListo*™ stand and invert the rack with the tube 3~4 times gently until the beads tightly bind to magnet.
10. Without removing the tube from *MagListo*™ rack, pour the supernatant out and remove the remaining supernatant using paper towel by blotting.
11. Go to step 17 of “DNA Extraction from Plant” in page 8 and continue the clean-up process.

XIII. Troubleshooting guide

Comments and suggestions

Low yield of DNA

Buffers or other reagents may have been exposed to external changes or conditions that reduce its effectiveness. Please make sure that reagents were stored at room temperature at all times upon arrival and all reagent bottles were closed tightly, in order to preserve pH and stability, and to avoid contamination.

Please check if ethanol has been added to the Buffer ③ (1st Washing) and ④ (2nd Washing). After adding ethanol, mix the buffer well and always mark the buffer bottles as ethanol added. If not, the Washing Buffer remains concentrated and may wash away the adsorbed DNA.

The lysis may have been incomplete. The amount of time for complete lysis varies depending on the type of tissue or sample used. A shaking water bath should be used for efficient lysis. Please, ensure that the starting material is completely disrupted. It would be helpful to increase the incubation time or reduce the amount of starting material.

Low purity

Beads may have been dried insufficiently. You must always completely remove the remaining washing solution and dry beads in a drying step. Remained ethanol can decrease the purity of DNA. Take enough time to completely dry the beads.

Incomplete suspension of beads during the washing step causes salts to remain in the purified DNA. Make the beads suspended thoroughly during the washing process.

Insufficient centrifugation causes remaining debris and precipitates in lysate. Any cell debris or precipitates should be removed before addition of magnetic nano bead. In this case, Increase centrifugation speed and time.

**Aggregation of Magnetic
Nano Bead**

There is an excess amount of sample to extract DNA. Appropriate amount of starting material (see “product specification” in page 4) should be used for efficient extraction of genomic DNA.

**There is a white precipitate
in some buffer**

A white precipitate may form in Buffer ① (Lysis) or Buffer ③ (1st Washing) after prolonged storage at low temperature. Incubating at 60°C should dissolve any precipitate in Buffer ① (Lysis) or Buffer ③ (1st Washing).

XIV. Ordering Information

Cat no.	Product Description	Size
K-3601SM	<i>MagListo</i> ™ 5M Plamid Extraction Kit, 8 rxn (mini)	1 kit
K-3601	<i>MagListo</i> ™ 5M Plamid Extraction Kit, 100 rxn (mini)	1 kit
K-3600	<i>MagListo</i> ™ 5M Plamid Extraction Kit, 500 rxn (mini)	1 kit
K-3602	<i>MagListo</i> ™ 5M Genomic DNA Extraction Kit, 8 rxn (mini)	1 kit
K-3603	<i>MagListo</i> ™ 5M Genomic DNA Extraction Kit, 100 rxn (mini)	1 kit
K-3604	<i>MagListo</i> ™ 5M Plant Genomic DNA Extraction Kit, 8 rxn (mini)	1 kit
K-3605	<i>MagListo</i> ™ 5M Plant Genomic DNA Extraction Kit, 100 rxn (mini)	1 kit
K-3606	<i>MagListo</i> ™ 5M Gel Extraction Kit, 8 rxn (mini)	1 kit
K-3607	<i>MagListo</i> ™ 5M Gel Extraction Kit, 100 rxn (mini)	1 kit
K-3608	<i>MagListo</i> ™ 5M PCR Purification Kit, 8 rxn (mini)	1 kit
K-3609	<i>MagListo</i> ™ 5M PCR Purification Kit, 100 rxn (mini)	1 kit
K-3610	<i>MagListo</i> ™ 5M Cell Total RNA Extraction Kit, 8 rxn (mini)	1 kit
K-3611	<i>MagListo</i> ™ 5M Cell Total RNA Extraction Kit, 100 rxn (mini)	1 kit
TM-1000	<i>MagListo</i> ™-8Ch Magnetic Separation Rack	1 ml tube x 8 holes
TM-1010	<i>MagListo</i> ™-2 Magnetic Separation Rack	2 ml tube x 8 holes
TM-1020	<i>MagListo</i> ™-15 Magnetic Separation Rack	15 ml tube x 6 holes
TM-1030	<i>MagListo</i> ™-50 Magnetic Separation Rack	15 ml tube x 6 holes
TM-1040	<i>MagListo</i> ™-96 Magnetic Separation Rack	96-well plate 1ea
TM-1100	<i>MagListo</i> ™ Magnetic Separation Rack Bundle Set	Each of <i>MagListo</i> ™-2, -15 ml, -50 ml, and -96 well (4 racks)
K-3601-A	Blow Dryer	1 ea
HT-15-NG	1.5 ml microcentrifuge tube	500 ea / pk
HT-20-NG	2 ml microcentrifuge tube	500 ea / pk

XIV. Explanation Symbols

	Catalog Number		Contains sufficient for (n) tests		USE BY		Consult Instruction For Use
	Batch code		Caution, consult accompanying documents		Temperature Limitation		In Vitro Diagnostics Medical Device
	Manufacturer		Caution, Potential Biohazard		DO NOT REUSE		Authorized Representative in the European Community

● Bioneer Worldwide

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