USER GUIDE

EcoQprep™ Plant Genomic DNA Kit (V1)

Cat. No. K-3702

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EcoQprep™ Plant Genomic DNA Kit (V1)

Kit for the extraction of genomic DNA from plants

User Guide

K-3702

Σ 50

Version No.: 0 (2025-06-04)

Please read all the information in booklet before using the unit

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Intended Use

EcoQprep™ Plant Genomic DNA Kit (V1) 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

EcoQprep™ and its kits are protected by the patents KR10-2344395.

Trademark

EcoQprep™ is a trademark of BIONEER Corporation.

Copyright

Notice

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Product Information

Components

Components	Cat. No.	Amount*	Storage
Proteinase K powder, lyophilized	KB-0111	25 mg x 1 ea	Refer to the “Storage” below.
RNase A powder, lyophilized	KB-3101	24 mg x 1 ea	
Magnetic Nano Bead-DNA	KB-7012	6 mL x 1 ea	Store at room temperature (15-25°C).
PL Buffer (Tissue Lysis)	KB-1032	20 mL x 1 ea	
PC Buffer (Binding)	KB-2025	15 mL x 1 ea	
PWM1 Buffer (1st Washing)	KB-3072	25 mL x 2 ea	
WB2 Buffer (2nd Washing)	KB-4018C	10 mL x 1 ea	
WE Buffer (3rd Washing)	KB-5016	40 mL x 1 ea	
EA Buffer (Elution)	KB-6012	25 mL x 1 ea	
1.5 mL Tube	KA-1100	50 ea x 1 pack	
One Page Protocol	-	1 ea	

* Mini – 50 rxn, Midi – 7 rxn, Maxi – 3 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 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

Starting material & DNA extraction efficiency

	Amount of Starting Sample		
Sample Type	**Mini**	**Midi**	**Maxi**
Plant tissue	< 100 mg	< 500 mg	< 1,000 mg
Seed	< 50 mg	< 250 mg	< 500 mg
Elution volume	100 µL	500 µL	1 mL
Typical DNA Yield	5-15 µg	15-80 µg	30-150 µg
DNA purity	A_{260}/A_{280} > 1.7		

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

Amount of starting sample

It is recommended to use 100 mg (leaf sample) and 50 mg (seed sample) as staring sample for EcoQprep™ Plant Genomic DNA Kit (V1) (mini scale).

Precautions

Take appropriate laboratory safety precautions and wear gloves when handling because PWM1 Buffer contains chaotropic salts which are irritants.

Introduction

Product Description

EcoQprep™ Plant Genomic DNA Kit (V1) is designed for extraction of highly purified total DNA from plant tissue of leaf and seed. The kit employs Magnetic Nano Beads to extract genomic DNA with the aid of EcoQprep™ Magnetic Separation Rack (Cat. No. TM-1012, TM-1021, TM-1031). The use of EcoQprep™ Magnetic Separation Rack along with the kit greatly increases user convenience by shortening the extraction time without centrifugation. 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

EcoQprep™ Plant Genomic DNA Kit (V1) is designed for extraction of genomic DNA from plant tissue of leaf and seed. 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.

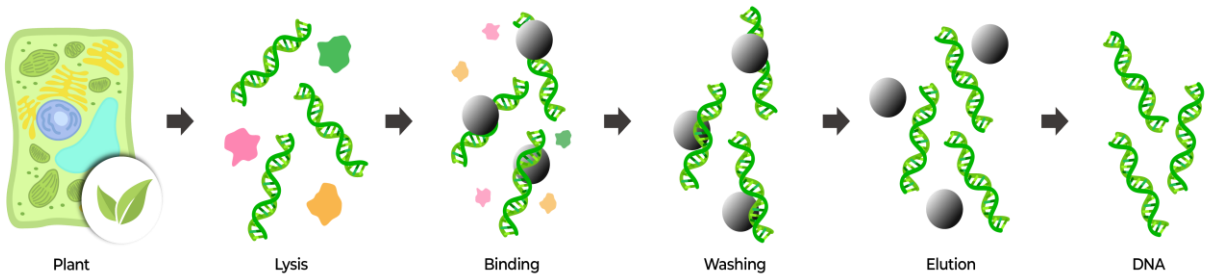


Figure 1. Genomic DNA extraction using silica-coated magnetic nanobeads.

Features & Benefits

- Comprehensive: High quality and yield of genomic DNA extraction from various samples such as leaves, stem, roots, or seeds.
- Convenient: Broad coverage of scales for mini, midi, and maxi isolation protocols with just a single kit.
- Rapid: Magnetic Nano Beads enable the rapid nucleic acid 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.

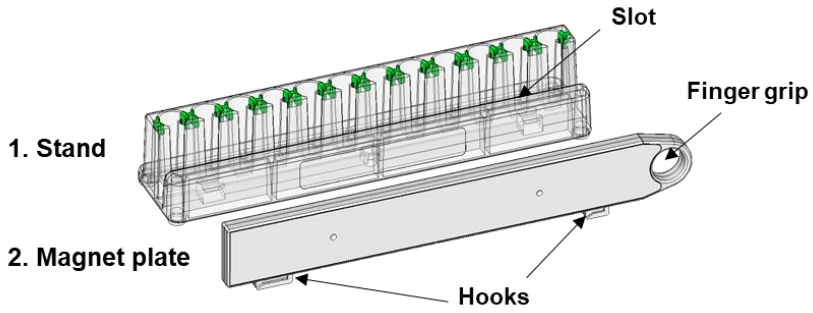
Features & Benefits

- 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.

EcoQprep™ Magnetic Separation Rack

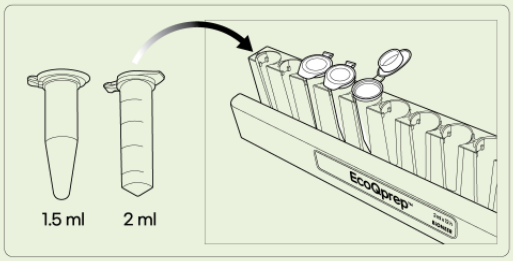
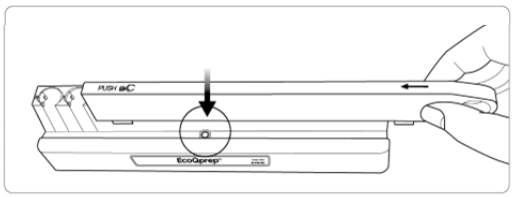
EcoQprep™ Magnetic Separation Rack (Cat. No. TM-1012) is designed for a fast and easy separation of the Magnetic Nano Beads. BIONEER offers
for 1.5 or 2 mL tube (Cat. No. TM-1012), 15 mL tube (Cat. No. TM-1021), 50 mL tube (Cat. No. TM-1031). These racks consisting of different size allow user to choose the product according to their needs.

Components of EcoQprep™ Magnetic Separation Rack



1. **Stand**: Holds up to 12 tubes with a non-slip design.
2. **Magnet plate**: Detachable from the stand's slot and contains a magnet.

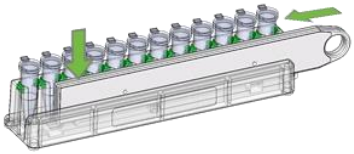
The following is recommended when using the EcoQprep™ Magnetic Separation Rack.

Precautions for Use	
	Confirm the orientation of the tube and the Rack.
	Ensure that the front hook of the Magnet plate overlaps more than half of the Stand.

1. **Attach the Magnet plate**

① Align the Magnet plate half of the Stand.

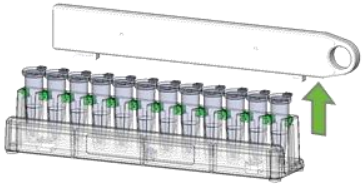
② Hold the finger grip of the Magnet plate and push.



2. **Detach the Magnet plate**

① Hold the finger grip of the Magnet plate and pull.

② Lift the Magnet plate.

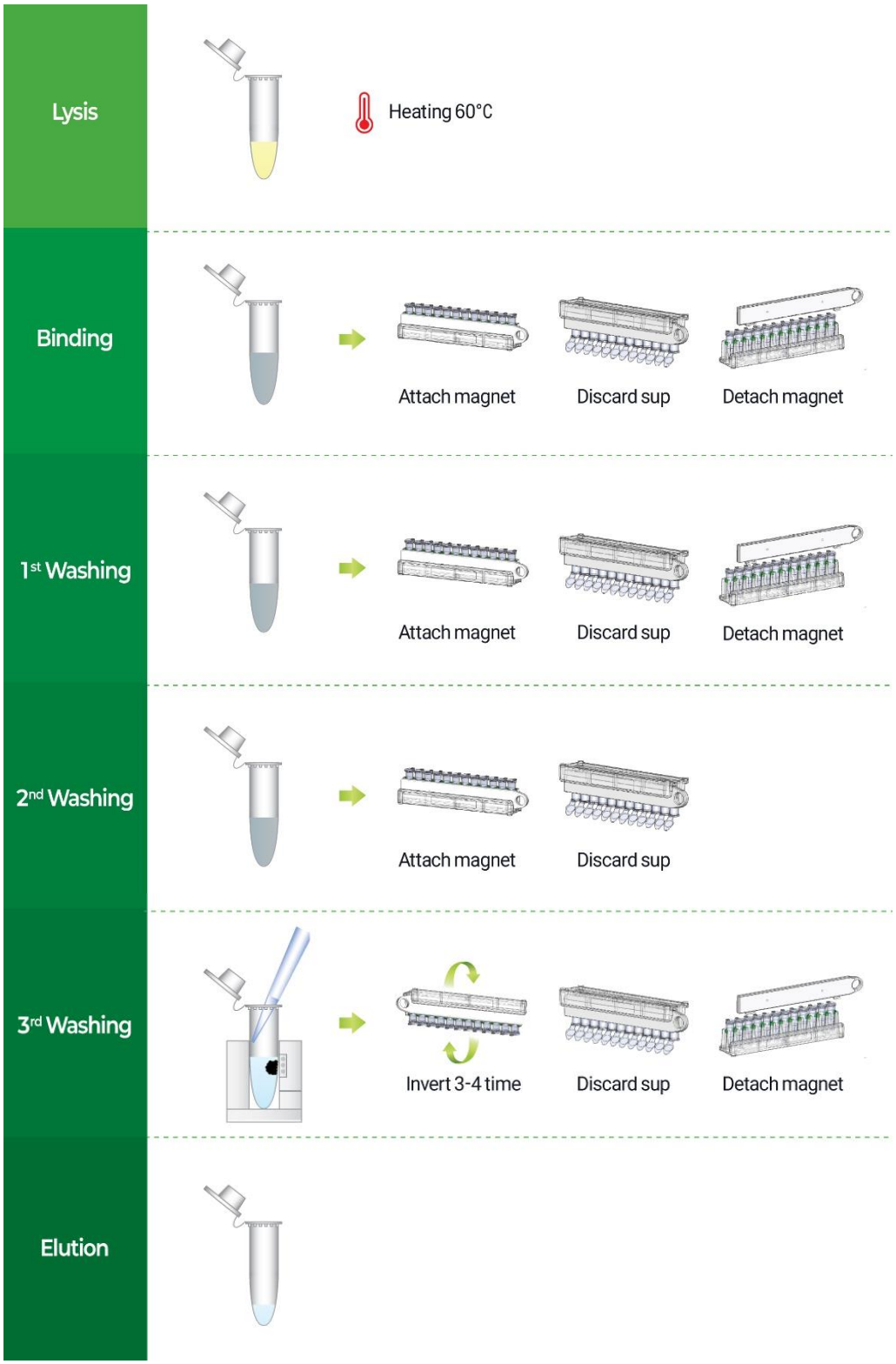


Features & Benefits of EcoQprep™ Magnetic Separation Rack

- Rapid: Isolates plasmid DNA, genomic DNA, and/or RNA quickly and economically.
  *Note: Extraction time varies depending on the product.
- 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. Freshly collected samples must be stored in a freezer or used immediately after collection. If the plant tissue sample is not to be used immediately after collection, it is recommended that it be stored in liquid nitrogen or at -70℃ to preserve its integrity. Please avoid repeated freezing and thawing.

Before Start

Before proceeding, please check the following:

1. Completely dissolve Proteinase K powder in 1,250 µL of DEPC-DW (Cat. No. C-9030) before use. Dissolved Proteinase K should be stored at 4°C for short-term storage, -20°C for long-term storage.

2. Completely dissolve RNase A powder in 600 µL DEPC-DW (Cat. No. C-9030) before use. Dissolved RNase A should be stored at 4°C for short-term storage, -20°C for long-term storage.

3. Pre-heat EA Buffer at 60°C before use.

4. Add specified volume of absolute ethanol (not provided) to PWM1 Buffer and WB2 Buffer, respectively before use (see bottle label).

5. Incubate the PL 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 Plants for Mini/Midi/Maxi Scale

1. **Sample preparing (Homogenization)**

   Grind (or homogenize) ≤ 100 mg (mini)/≤ 500 mg (midi)/≤ 1,000 mg (maxi) of tissue sample or ≤ 50 mg (mini)/≤ 250 mg (midi)/≤ 500 mg (maxi) of seed sample with a mortar and pestle (or homogenizer) and place them into the indicated clean tube below.

   1) (Mini) Place the homogenized tissue to a 1.5 mL or 2 mL tube.
   2) (Midi/Maxi) Place the homogenized tissue to a 15 mL tube.

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

2. **Lysis**

   1) Add 300 µL (mini)/1.5 mL (midi)/3 mL (maxi) of PL Buffer and 10 µL (mini)/75 µL (midi)/ 150 µL (maxi) of RNase A to the sample from step 1 and mix well by vortexing.
   2) Add 20 µL (mini)/100 µL (midi)/200 µL (maxi) of Proteinase K and mix well by vortexing.

      * **Note**: The sample should be completely immersed in the buffer.
   3) Incubate at 60°C for 10 minutes.

3. **Precipitation**

   1) Add 100 µL (mini)/500 µL (midi)/1 mL (maxi) of PC Buffer to the lysate and mix well by vortexing. This step precipitates detergent, proteins, and polysaccharides.
   2) Incubate for 5 minutes (mini)/10 minutes (midi, maxi) on ice.

4. **Precipitates removal**

   1) Centrifuge at 16,000 x g for 5 minutes (mini)/3,000 x g for 15 minutes (midi, maxi).
   2) Transfer the cleared supernatant to a new 1.5 mL or 2 mL tube (mini)/15 mL tube (midi, maxi).

5. **DNA precipitation**

   1) Add PWM1 buffer; 2X volume of supernatant.
   2) Mix well by vortexing.

6. DNA binding

1) 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.
2) Place the tube and attach the Magnet plate to the Stand of EcoQprep™ Magnetic Separation Rack 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) Discard the supernatant carefully without removing the tube from EcoQprep™ Magnetic Separation Rack. Completely remove the remaining supernatant using a paper towel by blotting.

*** Note:** Avoid strong impacts while blotting to prevent bead detachment.

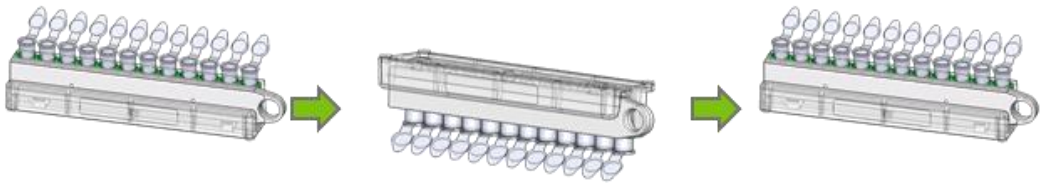


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the EcoQprep™ Magnetic Separation Rack. The non-slip design on stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

4) Detach the magnet plate from EcoQprep™ Magnetic Separation Rack.

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

7. 1st Washing

1) Add 500 µL (mini)/3 mL (midi)/5 mL (maxi) of PWM1 Buffer. Mix by vortexing until the beads are fully resuspended.
2) Repeat steps 6-2) to 6-4) to discard supernatant.

8. 2nd Washing

1) Add 700 µL (mini)/5 mL (midi)/10 mL (maxi) of WB2 Buffer. Mix thoroughly until the beads are fully resuspended.
2) Repeat steps 6-2) to 6-3) to discard supernatant.

   * **Note:** Do not detach the Magnet plate from the Rack.

9. 3rd Washing

1) Without removing the tubes from EcoQprep™ Magnetic Separation Rack, add 700 µL (mini)/5 mL (midi)/10 mL (maxi) of WE Buffer to the opposite side of beads.
2) Close the cap and invert the rack twice to remove ethanol from the sample.
3) Discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.
4) Detach the Magnet plate from EcoQprep™ Magnetic Separation Rack.

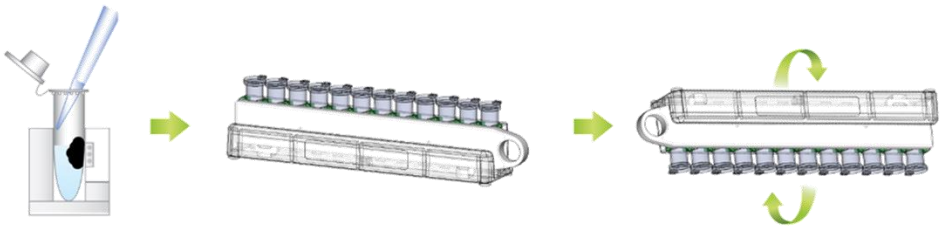


Figure 5. Washing the beads to remove residual ethanol.

* **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

1) Add 100 µL (mini)/500 µL (midi)/1 mL (maxi) of EA Buffer to each tube and resuspend DNA by vortexing or pipetting.
2) Incubate tubes at 60°C for at least 1 minute, and then vortex thoroughly.
3) Attach the Magnet plate and carefully transfer the supernatant containing DNA to a new tube.

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

DNA Clean-Up

DNA clean-up after nucleic acid extraction from plants is performed to remove impurities and improve accuracy in experiments requiring pure DNA, such as PCR, genetic transformation, and DNA sequencing.

1. Sample preparing

1) Transfer the eluted DNA or enzyme reaction products into the indicated clean tube below.
   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) 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) Add PWM1 Buffer; 2X volume of the eluted DNA.
4) Mix well by vortexing.

2. DNA precipitation

1) Add absolute ethanol (not provided); 2X volume of the eluted DNA.
2) Mix well by vortexing.

3. DNA Binding

1) 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.
2) Place the tube and attach the Magnet plate to the Stand of EcoQprep™ Magnetic Separation Rack 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) Discard the supernatant carefully without removing the tube from EcoQprep™ Magnetic Separation Rack. Completely remove the remaining supernatant using a paper towel by blotting.

*** Note:** Take care to avoid strong impacts while blotting to prevent bead detachment.

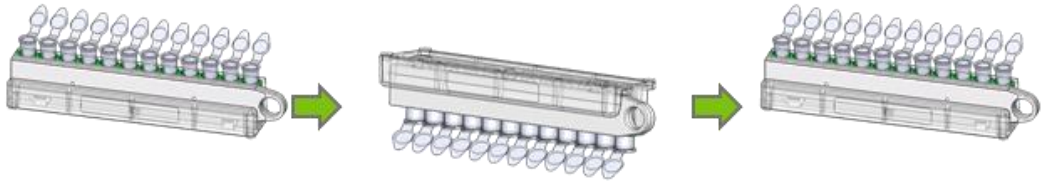


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the EcoQprep™ Magnetic Separation Rack. The non-slip design on stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

4) Detach the magnet plate from EcoQprep™ Magnetic Separation Rack.

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

4. 1st Washing

1) Add 700 µL (mini)/5 mL (midi)/10 mL (maxi) of WB2 Buffer. Mix by vortexing until the beads are fully resuspended.
2) Attach the Magnet plate and invert the rack gently 3-4 times until the beads bind tightly to the magnet.
3) Without removing the tubes from EcoQprep™ Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.
4) Go to step 9 of “DNA Extraction from Plants for Mini/Midi/Maxi Scale” on page 14.

Summary of Reagent Volumes Required in Each Step of DNA Extraction

DNA Extraction from Plant

Step	Buffer	Mini	Midi	Maxi
Sample	Plant Tissue	< 100 mg	< 500 mg	< 1,000 mg
	Plant Seed	< 50 mg	< 250 mg	< 500 mg
Lysis	PL Buffer	300 µL	1.5 mL	3 mL
Precipitation	PC Buffer	100 µL	500 µL	1 mL
DNA precipitation	PWM1 Buffer	2 volumes of supernatant		
DNA Binding	Magnetic Nano Bead	100 µL	500 µL	1 mL
1st Washing	PWM1 Buffer	500 µL	3 mL	5 mL
2nd Washing	WB2 Buffer	700 µL	5 mL	10 mL
3rd Washing	WE Buffer	700 µL	5 mL	10 mL
Elution	EA Buffer	100 µL	500 µL	1 mL
Tube type		1.5 or 2 mL tube	15 mL tube	15 mL tube

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, incubate the sample using a shaking water bath or a rocking platform.
	• **Excess starting material used.** Excess starting material can lead to incomplete lysis and neutralization. Use the appropriate amount of sample to ensure efficient genomic DNA extraction. For details, see “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 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.
	• **Insufficient centrifugation causes debris and precipitates to remain in the lysate.** Increase centrifugation speed and time. Any cell debris or precipitates should be removed before adding magnetic nano bead.
Aggregation of Magnetic Nano Beads	• **Excess starting material used.** Add appropriate amount of starting material. For more information, refer to “Specifications” on page 2.
Presence of a white precipitates in some buffers	• **PL Buffer and PWM1 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.
EcoQprep™ Plant Genomic DNA Kit (V1)	50 reactions	K-3702
EcoQprep™ Plant Genomic DNA Kit (V1)	200 reactions	K-3702-1

Related Products

Description	Cat. No.
Proteinase K Powder	KB-0111
RNase A Powder	KB-3101
EcoQprep™ Magnetic Separation Rack (2 mL)	TM-1012
EcoQprep™ Magnetic Separation Rack (15 mL)	TM-1021
EcoQprep™ Magnetic Separation Rack (50 mL)	TM-1031
DEPC-DW	C-9030

Explanation of Symbols

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

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