USER GUIDE

EcoQprep™ Genomic DNA Kit

Cat. No. K-3701

BIONEER
Innovation • Value • Discovery

EcoQprep™ Genomic DNA Kit

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

User Guide

K-3701

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™ Genomic DNA 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

EcoQprep™ and its kits are protected by the patents KR 10-2344395.

Trademark

EcoQprep™ is a trademark of BIONEER Corporation.

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Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

Components	Cat. No	K-3701 (50 rxn)*	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	
Poly(A), lyophilized	KB-0123	1 mg x 1 ea	
Magnetic Nanobead	KB-7012	6 mL x 1 ea	Store at room temperature (15-25°C).
TL Buffer	KB-1022	15 mL x 1 ea	
GB Buffer	KB-2043	15 mL x 1 ea	
WM1 Buffer	KB-3035	30 mL x 1 ea	
WB2 Buffer	KB-4018C	10 mL x 1 ea	
WE Buffer	KB-5016	40 mL x 1 ea	
EA Buffer	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.

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

Starting materials and DNA extraction efficiency

Scale		Micro	Mini	Midi	Maxi
Whole blood	Amount of Starting Sample	100 µL	200 µL	2 mL	4 mL
	Typical DNA Yield	< 5 µg	< 10 µg	< 80 µg	< 150 µg
Cultured cells	Amount of Starting Sample	~ 1 x 10^4 cells	~ 1 x 10^6 cells	~ 5 x 10^6 cells	~ 1 x 10^7 cells
	Typical DNA Yield	<120 ng	< 12 µg	< 60 µg	< 120 µg
Animal tissues	Amount of Starting Sample	~ 10 mg	~ 25 mg	~ 100 mg	~ 250 mg
	Typical DNA Yield	< 5 µg	< 10 µg	< 40 µg	< 120 µg
Bacterial cells (Gram (-), (+))	Amount of Starting Sample	-	~ 1 x 10^9 cells	~ 5 x 10^9 cells	~ 1 x 10^{10} cells
	Typical DNA Yield	-	< 15 µg	< 80 µg	< 150 µg
DNA Purity		A_{260}/A_{280} > 1.8			
Turnaround Time		>5 min	>5 min	>10 min	>15 min

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

Various kinds of forensic samples		
Amount of Starting sample	Dried body fluid spot or fingerprint (FTA card, paper, cloth, etc)	~7 mm or ~2 cm^2
	Hair	1 cm
	Bone and teeth	~ 100 mg
	Chewing gum	~ 30 mg
	Cigarette butts	~ 2 cm^2
	Buccal swab	Single piece of swab
	Urine	~15 mL
	Saliva	1-100 µL
DNA Yield	~ 5 µg	
DNA Purity	A_{260}/A_{280} > 1.8	
Turnaround Time	~ 10 min	

†Forensic samples: For more details, refer to page 12.

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

Micro scale extraction of genomic DNA from small amount of sample

EcoQprep™ 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 µL of Poly(A) (carrier RNA) to the starting sample. Carrier RNA can be removed later by RNase digestion.

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 x 10^6
12-well	4	4 x 10^5
24-well	2	2 x 10^5
48-well	1	1 x 10^5
96-well	0.35-0.6	4 x 10^4
Dishes		
35 mm	8	1.2 x 10^6
60 mm	21	3 x 10^6
100 mm	55	8 x 10^6
150 mm	148	2 x 10^7
Flasks		
50 mL	25	2.5 x 10^6
300 mL	75	1 x 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

EcoQprep™ Genomic DNA Kit is designed for extraction of highly purified total DNA from whole blood, animal tissues, cultured cells, bacterial cells, or forensic samples. 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) and *ExiPrep*™ 96 Lite (Cat. No. A-5250). The use of EcoQprep™ 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, and Short Tandem Repeat (STR) analysis.

Principle

EcoQprep™ Genomic DNA 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.

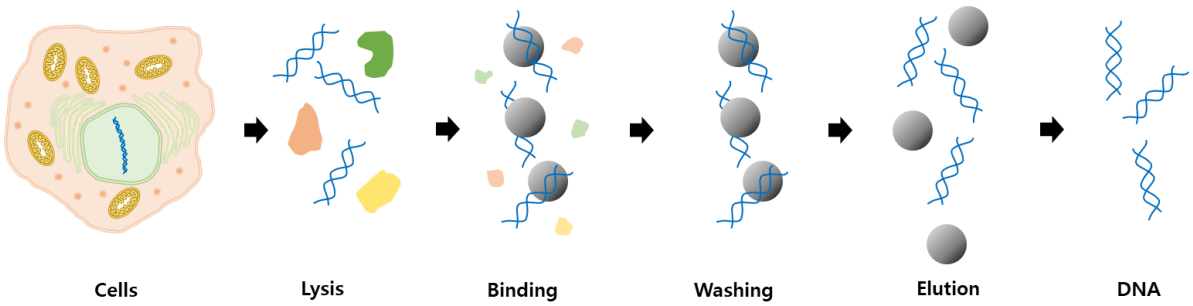


Figure 1. Genomic DNA extraction using silica-coated magnetic nanobeads.

Features & Benefits

- Comprehensive: High quality and yield of genomic DNA extraction from various samples such as whole blood, animal tissues, cultured cells, bacterial cells, or forensic samples such as saliva, urine, fingerprints, hair, nails, or bones.
- 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 (Mini: ~ 5 min, Midi: ~10 min, Maxi: ~15 min; time excludes sample pre-treatment).
- Efficient: A wide range of possible sample sizes even with a cultured 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 of Magnetic Nano Beads

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.

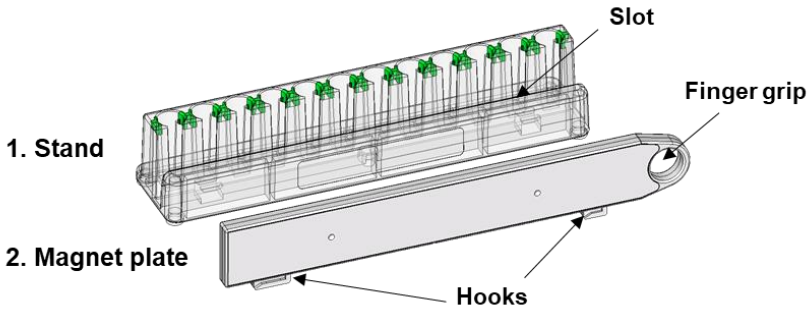
Features & Benefits of Magnetic Nano Beads

- Rapid: Fast binding guarantees high-throughput automation.
- Effectiveness: Large surface area enables more sensitive assays.
- Specificity: Globular structure increases specificity by reducing 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 various racks of different sizes 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

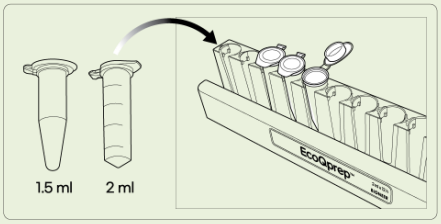
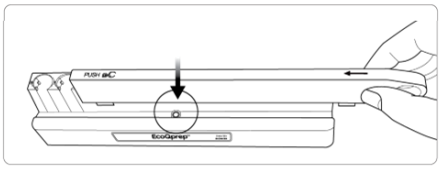


- Stand: Holds up to 12 tubes with a non-slip design.
- Magnet plate: Detachable from the stand's slot and contains a magnet.

Features & Benefits of EcoQprep™ Magnetic Separation Rack

- Rapid: Isolates plasmid DNA, genomic DNA, and/or RNA quickly and economically.
- Convenient: Simply disposes waste solution just by flipping the rack without centrifugation or pipetting as the silicon fixture secures the tube.

The followings are 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

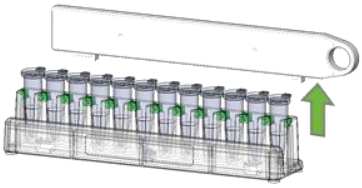
1) Align the Magnet plate half of the Stand.

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

2. Detach the Magnet plate

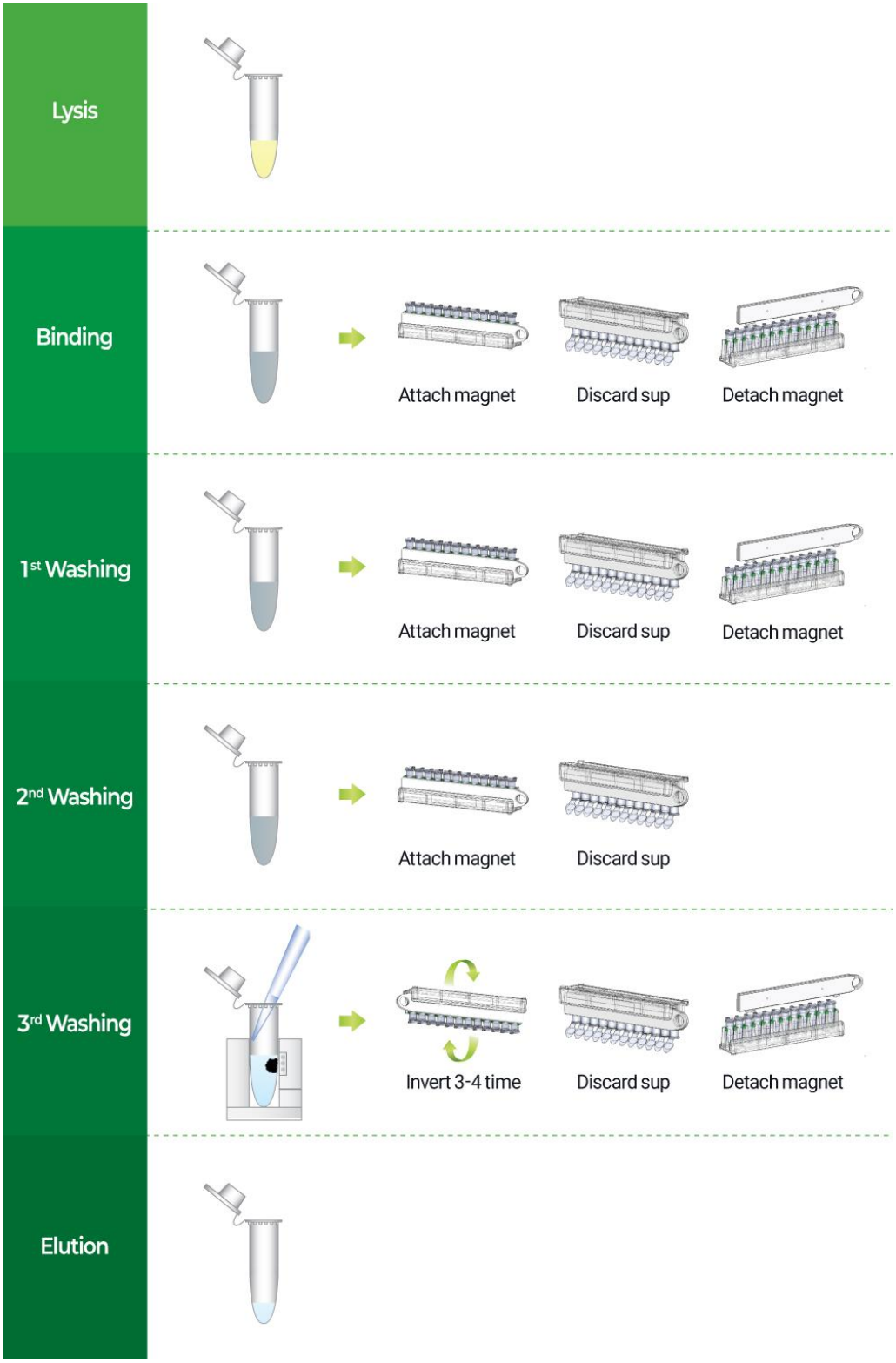
1) Hold the finger grip of the Magnet plate and pull.

2) Lift the Magnet plate



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.

Animal Tissue

Animal 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.

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 EcoQprep™ Genomic DNA 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.

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.

Pretreatment of Forensic 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.

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, BIONEER) or nuclease-free water 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 of DEPC-DW (Cat. No.C-9030, BIONEER) or nuclease-free water before use. Dissolved RNase A should be stored at 4°C for short-term storage, -20°C for long-term storage.

3. Completely dissolve Poly(A) in 1,000 µL of DEPC-DW (Cat. No.C-9030, BIONEER) or nuclease-free water before use. Dissolved Poly(A) should be stored at 4°C for short-term storage, -20°C for long-term storage.

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

5. Add specified volume of absolute ethanol (not provided) to WM1 Buffer and WB2 Buffer, respectively before use (see bottle label).

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

7. 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.

Protocol Ⅰ : DNA Extraction from Whole Blood

Micro/Mini/Midi/Maxi Scale

Step	Buffer	Micro	Mini	Midi	Maxi
Sample preparation	Sample	100 µL	200 µL	2 mL	4 mL
Lysis	Proteinase K	10 µL	20 µL	100 µL	200 µL
	GB Buffer	100 µL	200 µL	2 mL	4 mL
Precipitation	Absolute ethanol (not provided)	200 µL	400 µL	4 mL	8 mL
Binding	Magnetic Nano Bead	100 µL	100 µL	500 µL	1 mL
1st washing	WM1 Buffer	500 µL *2	500 µL *2	3 mL *2	5 mL *2
2nd washing	WB2 Buffer	700 µL	700 µL	5 mL	10 mL
3rd washing	WE Buffer	700 µL	700 µL	5 mL	10 mL
Elution	EA Buffer	50 µL	100 µL	500 µL	1 mL
Tube type		1.5 or 2 mL tube	1.5 or 2 mL tube	15 mL tube	50 mL tube

1. Sample preparation

1) Apply 100 µL (micro)/ 200 µL (mini)/ 2 mL (midi)/ 4 mL (maxi) of whole blood or buffy coat sample.

* **Note:** If the sample volume is less than the indicated volume above, adjust the total volume to 100 µL (micro)/200 µL (mini)/ 2 mL (midi)/ 4 mL (maxi) by adding PBS buffer (not provided, Cat. No. C-9024, BIONEER).

2. Lysis

1) Add 10 µL (micro)/ 20 µL (mini)/ 100 µL (midi)/ 200 µL (maxi) of Proteinase K to the sample.
2) Add 100 µL (micro)/ 200 µL (mini)/ 2 mL (midi)/ 4 mL (maxi) of GB Buffer to the sample and mix well by vortexing.

* **Note:** Ensure complete resuspension of the sample to maximize lysis efficiency.

3) Incubate at 60°C for 10 minutes.

3. DNA precipitation

1) Add 200 µL (micro)/ 400 µL (mini)/ 4 mL (midi)/ 8 mL (maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting.

4. DNA binding

1) 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.

***Note**: Resuspend the Magnetic Nano Beads by thorough mixing immediately before use.

2) 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.

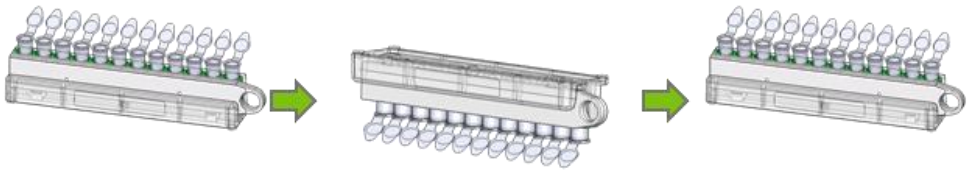


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the EcoQprep™ Magnetic Separation Rack. The non-slip design on stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

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.

5. 1st Washing

1) Add 500 µL (micro, mini)/ 3 mL (midi)/ 5 mL (maxi) of WM1 Buffer. Mix by vortexing until the beads are fully resuspended.
2) Proceed with steps 4-2) to 4-3) to discard the supernatant.
3) Detach the Magnet plate.
4) Repeat steps 5-1) to 5-3) once more for additional washing.

6. 2nd Washing

1) Add 700 µL (micro, mini)/5 mL (midi)/10 mL (maxi) of WB2 Buffer. Mix thoroughly until the beads are fully resuspended.
2) Proceed with steps 4-2) to 4-3) to discard the supernatant.

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

7. 3rd Washing

1) Without removing the tubes from EcoQprep™ Magnetic Separation Rack, add 700 µL (micro, mini)/ 5 mL (midi)/ 10 mL (maxi) of WE Buffer to the opposite side of beads. Close the cap and invert the rack twice to remove ethanol from the sample.

*** 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.

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

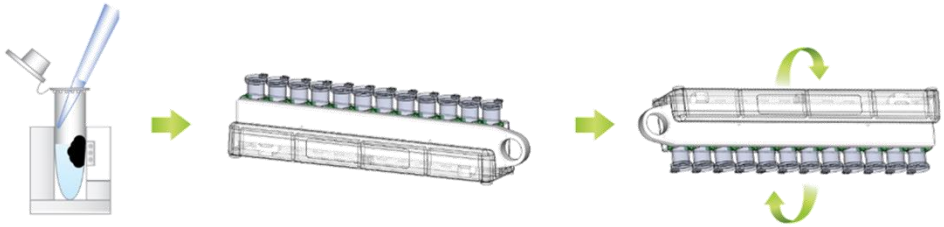


Figure 5. Washing the beads to remove residual ethanol.

3) Detach the Magnet plate from EcoQprep™ Magnetic Separation Rack.

8. Elution

1) 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.
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.

Protocol Ⅱ: DNA Extraction from Cultured Cells

Micro/Mini/Midi/Maxi Scale

Step	Buffer	Micro	Mini	Midi	Maxi
Sample preparing	Cultured Cells	~ 1 x 10^4	~ 1 x 10^6	~ 5 x 10^6	~ 1 x 10^7
	PBS (Not provided)	100 µL	200 µL	1 mL	1 mL
	Poly(A)	4 µL	-	-	-
Lysis	Proteinase K	10 µL	20 µL	100 µL	200 µL
	RNase A	2 µL	10 µL	75 µL	150 µL
	GB Buffer	100 µL	200 µL	1 mL	1 mL
Precipitation	Absolute ethanol (not provided)	200 µL	400 µL	2 mL	2 mL
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	WB2 Buffer	700 µL	700 µL	5 mL	10 mL
3rd washing	WE Buffer	700 µL	700 µL	5 mL	10 mL
Elution	EA Buffer	50 µL	100 µL	500 µL	1 mL
Tube type		1.5 or 2 mL tube	1.5 or 2 mL tube	15 mL tube	15 mL tube

1. Sample preparation

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.
   A. (Micro/ Mini) Transfer the resuspended cells to a 1.5 mL or 2 mL tube.
   B. (Midi/ Maxi) Transfer the resuspended cells to a 15 mL tube.
3) If the sample contains low number of cells (~1 x 10^4 cells), add 4 µL (micro) of Poly (A) to the sample.

2. Lysis

1) Add 10 µL (micro)/ 20 µL (mini)/ 100 µL (midi)/ 200 µL (maxi) of Proteinase K to the sample.
2) If RNA-free genomic DNA is required, add 2 µL (micro)/ 10 µL (mini)/ 75 µL (midi)/ 150 µL (maxi) of RNase A to the sample and mix well by vortexing.
3) Incubate for 2 minutes at room temperature.
4) Add 100 µL (micro)/ 200 µL (mini)/ 1 mL (midi, maxi) of GB Buffer to the sample and mix well by vortexing.

* **Note:** Ensure complete resuspension of the sample to maximize lysis efficiency.

5) Incubate at 60°C for 10 minutes.

3. DNA precipitation

1) Add 200 µL (micro)/ 400 µL (mini)/ 2 mL (midi, maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting.

4. DNA binding

1) 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.
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.

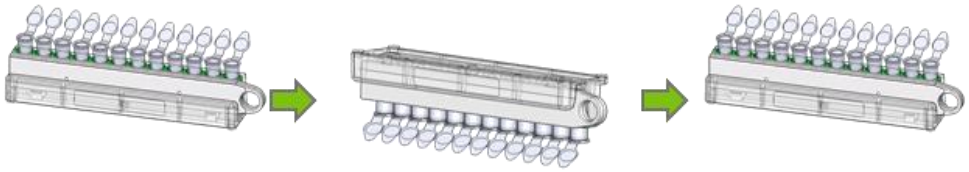


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the EcoQprep™ Magnetic Separation Rack. The non-slip design on stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

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.

5. 1st Washing

1) Add 700 µL (micro, mini)/ 5 mL (midi)/ 10 mL (maxi) of WM1 Buffer. Mix by vortexing until the beads are fully resuspended.
2) Proceed with steps 4-2) to 4-3) to discard the supernatant.
3) Detach the Magnet plate.

6. 2nd Washing

1) Add 700 µL (micro, mini)/5 mL (midi)/10 mL (maxi) of WB2 Buffer. Mix thoroughly until the beads are fully resuspended.
2) Proceed with steps 4-2) to4-3) to discard supernatant.

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

7. 3rd Washing

1) Without removing the tubes from EcoQprep™ Magnetic Separation Rack, add 700 µL (micro, mini)/ 5 mL (midi)/ 10 mL (maxi) of WE Buffer to the opposite side of beads. Close the cap and invert the rack twice to remove ethanol from the sample.

   * **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.

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

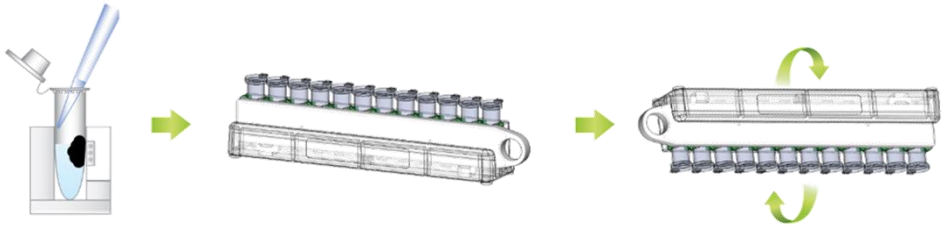


Figure 5. Washing the beads to remove residual ethanol.

3) Detach the Magnet plate from EcoQprep™ Magnetic Separation Rack.

8. Elution

1) 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.
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.

Protocol Ⅲ: DNA Extraction from Animal Tissue

Micro/Mini/Midi/Maxi Scale

Step	Buffer	Micro	Mini	Midi	Maxi
Sample preparing	Animal Tissue	~ 10 mg	~ 25 mg	~ 100 mg	~ 250 mg
Lysis	TL Buffer	90 µL	180 µL	1.8 mL	3.6 mL
	Proteinase K	10 µL	20 µL	100 µL	200 µL
	RNase A	5 µL	10 µL	75 µL	150 µL
	GB Buffer	100 µL	200 µL	2 mL	4 mL
Precipitation	Absolute ethanol (not provided)	200 µL	400 µL	4 mL	8 mL
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	WB2 Buffer	700 µL	700 µL	5 mL	10 mL
3rd washing	WE Buffer	700 µL	700 µL	5 mL	10 mL
Elution	EA Buffer	100 µL	100 µL	500 µL	1 mL
Tube type		1.5 or 2 mL tube	1.5 or 2 mL tube	15 mL tube	50 mL tube

1. Sample preparation (Homogenization)

1) Grind (or homogenize) ~ 10 mg (micro)/ ~ 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†.

   A. (Micro, Mini) Transfer the homogenized tissue sample to a 1.5 mL or 2 mL tube.

   B. (Midi) Transfer the homogenized tissue sample to a 15 mL tube.

   C. (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

1) Add 90 µL (micro)/ 180 µL (mini)/ 1.8 mL (midi)/ 3.6 mL (maxi) of TL Buffer.
2) Add 10 µL (micro)/20 µL (mini)/ 100 µL (midi)/ 200 µL (maxi) of Proteinase K to the sample and mix well by vortexing.
3) If RNA-free genomic DNA is required, add 5 µL (micro)/ 10 µL (mini)/ 75 µL (midi)/ 150 µL (maxi) of RNase A to the sample and mix well by vortexing.
4) Incubate for 2 minutes at room temperature.
5) Incubate at 60°C until the sample has been completely lysis.
6) Add 100 µL (micro)/200 µL (mini)/ 2 mL (midi)/ 4 mL (maxi) of GB Buffer to the sample and mix well by vortexing.

* **Note:** Ensure complete resuspension of the sample to maximize lysis efficiency.

3. DNA precipitation

1) Add 200 µL (micro)/400 µL (mini)/ 4 mL (midi)/ 8 mL (maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting.
2) Go to step **4. DNA binding** of **"Protocol II : DNA Extraction from Cultured Cells"** on **page 18**.

ProtocolIV: DNA Extraction from Bacterial Cells (Gram-Negative Bacteria)

Mini/Midi/Maxi Scale				
Step	**Buffer**	**Mini**	**Midi**	**Maxi**
Sample preparing	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
	Proteinase K	20 µL	100 µL	200 µL
	RNase A	10 µL	75 µL	150 µL
	GB Buffer	200 µL	2 mL	4 mL
Precipitation	Absolute ethanol (not provided)	400 µL	4 mL	8 mL
Binding	Magnetic Nano Bead	100 µL	500 µL	1 mL
1st washing	WM1 Buffer	700 µL	5 mL	10 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	50 mL tube

1. Sample preparation

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.
   A. (Mini) Transfer the homogenized tissue sample to a 1.5 mL or 2 mL tube.
   B. (Midi) Transfer the homogenized tissue sample to a 15 mL tube.
   C. (Maxi) Transfer the homogenized tissue sample to a 50 mL tube.

2. Lysis

1) Add 180 µL (mini)/ 1.8 mL (midi)/ 3.6 mL (maxi) of TL Buffer.
2) Add 20 µL (mini)/ 100 µL (midi)/ 200 µL (maxi) of Proteinase K to the sample and mix well by vortexing.
3) If RNA-free genomic DNA is required, add 10 µL (mini)/ 75 µL (midi)/ 150 µL (maxi) of RNase A to the sample and mix well by vortexing.
4) 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:** Ensure complete resuspension of the sample to maximize lysis efficiency.

3. DNA precipitation

1) Add 400 µL (mini)/ 4 mL (midi)/ 8 mL (maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting.
2) Go to step **4. DNA binding** of **"Protocol II : DNA Extraction from Cultured Cells"** on **page 18**.

Protocol V : DNA Extraction from Bacterial Cells (Gram-Positive Bacteria)

Mini/Midi/Maxi Scale				
Step	**Buffer**	**Mini**	**Midi**	**Maxi**
Sample preparing	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
	RNase A	10 µL	75 µL	150 µL
	Proteinase K	20 µL	100 µL	200 µL
	GB Buffer	200 µL	2 mL	4 mL
Precipitation	Absolute ethanol (not provided)	400 µL	4 mL	8 mL
Binding	Magnetic Nano Bead	100 µL	500 µL	1 mL
1st washing	WM1 Buffer	700 µL	5 mL	10 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	50 mL tube

1. Sample preparation

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.

*** 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.

A. (Mini) Transfer the homogenized tissue sample to a 1.5 mL or 2 mL tube.
B. (Midi) Transfer the homogenized tissue sample to a 15 mL tube.
C. (Maxi) Transfer the homogenized tissue sample to a 50 mL tube.

2. Lysis

1) Add 20 µL (mini)/ 100 µL (midi)/ 200 µL (maxi) of lysozyme (100 mg/mL, not provided) and mix well by vortexing.
2) If RNA-free genomic DNA is required, add up to 10 µL (mini)/ 75 µL (midi)/ 200 µL (maxi) of RNase A to the sample and gently mix.
3) Incubate at 37°C for 30 minutes.
4) Add 20 µL (mini)/ 100 µL (midi)/ 200 µL (maxi) of Proteinase K and mix well by vortexing.
5) Add 200 µL (mini)/ 2 mL (midi)/ 4 mL (maxi) of GB Buffer and mix well by vortexing.
6) Incubate at 60°C for 30 minutes or until bacterial cells are completely lysed.

3. DNA precipitation

1) Add 400 µL (mini)/ 4 mL (midi)/ 8 mL (maxi) of absolute ethanol (not provided) to the lysate and mix well by pipetting.
2) Go to step **4. DNA binding** of **"Protocol II : DNA Extraction from Cultured Cells"** on **page 18**.

ProtocolⅥ: DNA Extraction from Various Forensic Samples

Step	Buffer	Forensic samples	Urine	Saliva
Sample preparing	-	Pretreated samples	~ 15 mL	1-100 µL
Lysis	Proteinase K	10 µL	10 µL	10 µL
	TL Buffer	300 µL	300 µL	-
	GB Buffer	300 µL	300 µL	100 µL
Precipitation	Absolute ethanol (not provided)	600 µL	600 µL	600 µL
Binding	Magnetic Nano Bead	100 µL	100 µL	100 µL
1st washing	WM1 Buffer	700 µL	700 µL	700 µL
2nd washing	WB2 Buffer	700 µL	700 µL	700 µL
3rd washing	WE Buffer	700 µL	700 µL	700 µL
Elution	EA Buffer	100 µL	100 µL	100 µL

DNA Extraction from Forensic samples (excluding Urine and Saliva)

1. Sample preparation

1) Prepare the forensic samples according to the pretreatment method on page 12.
2) Apply the collected samples to 1.5 mL tube.

2. Lysis

1) Add 10 µL of Proteinase K to the sample.
2) Add 300 µL TL Buffer 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, extend the incubation time up to overnight as needed.
5) Add 300 µL of GB Buffer to the lysate and mix well by vortexing.

   *** Note:** Ensure complete resuspension of the sample to maximize 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.

3. DNA precipitation

1) Add 600 µL of absolute ethanol (not provided) to the lysate and mix well by pipetting.
2) Go to step **4.DNA binding** of "**DNA Extraction from Cultured cells**" on **page 18**.

DNA Extraction from Urine

1. Sample preparation

1) Centrifuge the urine sample and discard the supernatant.
   A. (~2 mL) Centrifuge at 6,000 x g for 2 minutes.
   B. (~15 mL) Centrifuge at 2,000 x g for 10 minutes.
2) Resuspend the sample in 500 µL PBS Buffer (not provided, Cat. No C-9024) by vortexing or pipetting. Transfer the sample into a 1.5 mL or 2 mL tube.
3) Centrifuge at 6,000 x g for 2 minutes and discard the supernatant.

2. Lysis

1) Add 10 µL of Proteinase K to the sample.
2) Add 300 µL TL Buffer to the sample and mix well by vortexing.
3) **(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.
4) Incubate at 60°C for 1 hour.
5) Add 300 µL of GB Buffer to the sample and mix well by vortexing.
   * **Note:** Ensure complete resuspension of the sample to maximize lysis efficiency.

3. DNA precipitation

1) Add 600 µL of absolute ethanol (not provided) to the lysate and mix well by pipetting.
2) Go to step **4.DNA binding** of "**DNA Extraction from Cultured cells**" on **page 18**.

DNA Extraction from Saliva

1. Sample preparation

1) Apply 1-100 µL of 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. Lysis

1) Add 10 µL of Proteinase K to the sample.
2) Add 100 µL of GB Buffer to the sample and mix well by vortexing.

*** Note:** Ensure complete resuspension of the sample to maximize lysis efficiency.

3) Incubate at 60°C for 10 minutes.

3. DNA precipitation

1) Add 600 µL of absolute ethanol (not provided) to the lysate and mix well by pipetting.
2) Go to step **4.DNA binding** of "**DNA Extraction from Cultured cells**" on **page 18**.

ProtocolⅦ: DNA Clean-Up

1. Sample preparation

1) Transfer the eluted DNA or enzyme reaction products into the indicated clean tube below.
   A. (Micro/ 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. Lysis

1) 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
2) Incubate for 2 minutes at room temperature.
3) Add 1 volume of GB Buffer to 1 volume of the eluted DNA and mix well by vortexing.

3. DNA precipitation

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

4. DNA binding

1) 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.
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.

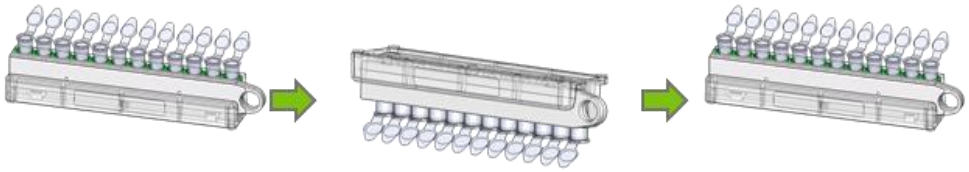


Figure 3. How to discard the supernatant. Discard the supernatant by inverting the EcoQprep™ Magnetic Separation Rack. The non-slip design on stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

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.

5. 1st Washing

1) Add 700 µL (micro, 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.

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

6. 2nd Washing

1) Add 700 µL (micro, mini)/ 5 mL (midi)/ 10 mL (maxi) of WE Buffer to the opposite side of beads. Close the cap and invert the rack twice to remove ethanol from the sample.
2) Discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

*** 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.

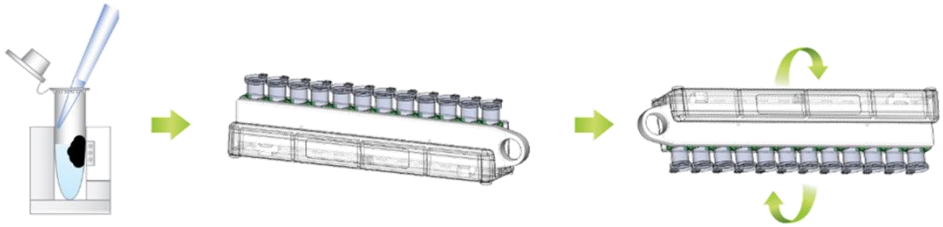


Figure 5. Washing the beads to remove residual ethanol.

3) Detach the Magnet plate from EcoQprep™ Magnetic Separation Rack.

7. Elution

1) 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.
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.

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. To achieve efficient lysis, perform a shaking water bath or use 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.** Ensure all Magnetic Nano Beads are securely bound to

	the magnet before discarding.
	• **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(Cat. No. KB-0101) to the sample before adding GB Buffer. For more information, refer to “DNA Clean-Up” on page 31.
Aggregation of Magnetic Nano Beads	• **Excess starting material used.** Use the appropriate amount of sample to ensure efficient genomic DNA extraction. For details, see “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
EcoQprep™ Genomic DNA Kit	50 reactions	K-3701

Related Products

Description	Cat. No
EcoQprep™ Magnetic Separation Rack (2 mL)	TM-1012
EcoQprep™ Magnetic Separation Rack (15 mL)	TM-1021
EcoQprep™ Magnetic Separation Rack (50 mL)	TM-1031
1M Tris-HCl (pH 8.0)	C-9006
0.5M EDTA (pH 8.0)	C-9007
Phosphate Buffered Saline (PBS)	C-9024
DEPC-DW	C-9030

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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