HiYield™ Genomic DNA Extraction Kit

Ver. 2024 - 3

ARROWTEC

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Content and Storage

Genomic DNA Extraction Kit Mini

Blood/Bacteria/Cultured Cells
Cat.No. YGB50 // YGB100

Store at room temperature 15 ℃~25 ℃

Kit Contents

Cat.No. YGB50
50 mini preps / kit

RBC Lysis Buffer.....100 ml
GB Buffer.....15 ml
GT Buffer.....15 ml
W1 Buffer.....25 ml
Wash Buffer (Concentrated).....25 ml*
Elution Buffer .....30 ml
*Proteinase K ** .....11 mg*
GD Column .....50 pcs
2 ml Collection Tube .....50 pcs

Cat.No. YGB100
100 mini preps / kit

RBC Lysis Buffer.....200 ml
GB Buffer.....30 ml
GT Buffer.....30 ml
W1 Buffer.....50 ml
Wash Buffer (Concentrated).....25 ml*
Elution Buffer .....30 ml
*Proteinase K ** .....11 mg x 2*
GD Column .....100 pcs
2 ml Collection Tube .....100 pcs

Sample (Protocols Included):
300 µl Whole Blood (up to 1ml),10^7 Cultured Cells,10^9 Bacteria (Gram +/-),10^7 Yeast.
Average Yields: *300µl Whole Blood - 6µg, 200 µl Buffy - 50µg, 5 x 10^6Lymph./Cult. Cells - 50µg.*

** Add 4 times volume of ethanol(96-100%) to Wash Buffer prior to initial use.*
*** Add 1.1 ml ddH2O to the tube and mix by vortexing. Store prepared Proteinase K (10mg/ml) at 4 ℃. For long term storage, aliquot and store at -20 ℃.*
Additional Requirement: *Microcentrifuge Tube, Ethanol (96-100%), RNase A (10 mg/ml).*

Content and Storage

Genomic DNA Extraction Kit Mini

Tissue/Bacteria/Cultured Cells
Cat.No. YGT50//YGT100

Store at room temperature 15℃~25℃

Kit Contents

Cat.No. YGT50
50 mini preps / kit

GB Buffer	30 ml
GT Buffer	15 ml
W1 Buffer	25 ml
Wash Buffer (Concentrated)*	25 ml
Elution Buffer	30 ml
Proteinase K **	11 mg
GD Column	50 pcs
2 ml Collection Tube	50 pcs
Micropestles	50 pcs

Cat.No. YGT100
100 mini preps / kit

GB Buffer	60 ml
GT Buffer	30 ml
W1 Buffer	50 ml
Wash Buffer (Concentrated)*	25 ml
Elution Buffer	30 ml
Proteinase K **	11 mg x 2
GD Column	100 pcs
2 ml Collection Tube	100 pcs
Micropestles	100 pcs

Sample (Protocols Included):
20mg of tissue, 0.5 cm of mouse tail, 10^7 Cultured Cells,10^9 Bacteria (Gram +/-), 10^7 Yeast, Tissue, Paraffin-Embedded Tissue, Buccal Swab

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** Add 4 times volume of ethanol (96-100%) to Wash Buffer prior to initial use.*
*** Add 1.1 ml ddH20 to the tube and mix by vortexing. Store prepared Proteinase K (10mg/ml) at 4 ℃. For long term storage ,aliquot and store at -20 ℃.*

Description

Genomic DNA Extraction Mini Kit provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood, plasma, serum, buffy coat, other body fluids, lymphocytes and cultured cells. This kit is also ideal for bacterial and cultured cells. The blood protocol utilises RBC lysis buffer, GB buffer and a rapid heating step to release DNA into solution. DNA in the chaotropic salt solution binds to the glass fiber matrix of column. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer or water. There is no requirement for phenol/chloroform extraction or alcohol precipitation. Purified DNA of approximately 20-30 kb is suitable for PCR or other enzymatic reactions.

Quality Control

The quality of Genomic DNA Mini Kit is tested on a lot-to-lot basis.
The kits are tested by isolation of genomic DNA from 200µl of human whole blood. The purified DNA is quantified by spectrophotometer and the yield of genomic DNA is 4-6µg with A260/A280 ratio 1.6-1.8.

Reference

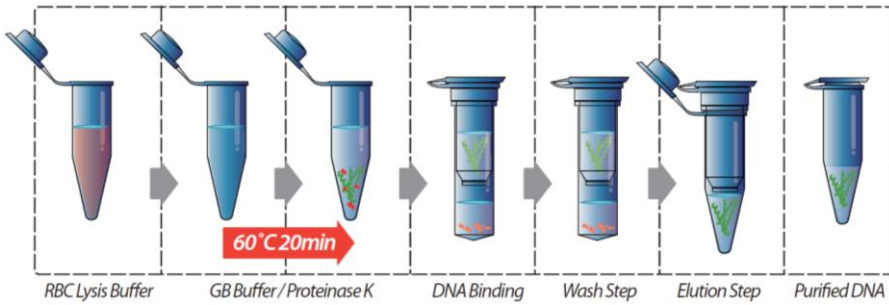
Vogelstein, B., and Gillespie , D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Note

* For research use only. Not for use in diagnostic or therapeutic procedures.
* Buffer contains guanidine hydrochloride which is harmful and can act as an irritant. During operation, always wear a lab coat, disposable gloves and protective goggles.

Blood/Bacteria/Cultured Cells Protocol

OverView



Fresh Blood Protocol

RBC Lysis Buffer is provided to remove non-nucleated red blood cells and reduce hemoglobin contamination. But when the blood sample is less than 50µl or sample consists of nucleated blood cells, the Cells Protocol is recommended to purify DNA.

1. Collect fresh blood in EDTA-Na2 treated collection tubes (or other anticoagulant mixture).
2. Apply up to 300µl of blood to a 1.5ml microcentrifuge tube. If blood sample is more than 300µl (up to 1 ml), apply the sample to a sterile 15ml centrifuge tube.
3. Add 3 times the sample volume of RBC Lysis Buffer and mix by inversion. **Do not vortex**.
4. Incubate the tube for 5 minutes at room temperature.
5. Centrifuge for 2 minutes at 3,000 x g and discard the supernatant.
6. Add 200µl RBC Lysis Buffer to resuspend the cell pellet.
7. Add 200µl GB Buffer and 20µl Proteinase K (10mg/ml) to the tube and mix by vortexing.
8. Incubate at 60˚C for 20 minutes until the sample lysate is clear. During incubation, invert the tube every 3 min.

At this time, preheat required Elution Buffer (200µl per sample) at 70 ˚C.DNA Elution.

9. proceed to **DNA Binding Step (page 10)**.

Frozen Blood Protocol

1. 200µl Blood + 20µl Proteinase K (10mg/ml) + 200µl GB Buffer, Incubate at 60°C for 30 min.

At this time, preheat required Elution Buffer (200µl per sample) at 70 °C.DNA Elution.

2. proceed to **DNA Binding Step (page 10)**.

Cultured Cells Protocol

Additional Requirement: *Microcentrifuge tube, Ethanol (96-100%), RNase A (10mg/ml)*

Sample Preparation

Cultured Animal Cells:

If using adherent cells, trypsinize the cells before harvesting.

1. Transfer 10^6-10^7 cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation at 2,000 rpm for 5 min.
2. Discard the supernatant and resuspend the cells in 150µl 1X RBC Buffer.

Nucleated Erythrocytes:

For nucleated erythrocytes (e.g. bird or fish), the sample volume can be up to 10µl.

1. Add 150µl GT Buffer to a microcentrifuge tube and apply blood sample to tube.
2. Vortex to mix sample.

3. Add 200µl GB Buffer to the sample. Vortex for 5 seconds to mix sample.
4. Incubate at 70°C for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

At this time, preheat required Elution Buffer (200µl per sample) at 70 °C.DNA Elution.

5. proceed to **DNA Binding Step (page 10).**

Bactrerial Protocol

Additional Requirements : *Lysozyme Buffer, Microcentrifuge tube, RNase A (10mg/ml)*

For Gram-negative Bacteria

1. Transfer bacterial culture (<10^9) to a microcentrifuge tube (not provided). Centrifuge for 1 min at 10,000 x g (13,000 rpm) and discard the supernatant.
2. Add 200µl of GT Buffer to the tube and vortex or pipette to resuspend the cell pellet. Incubate at room temperature for 5 minutes.

For Gram-positive Bacteria

Prepare Lysozyme Buffer: *(20mg/ml lysozyme; 20mM Tris-HCl; 2mM EDTA; 1% Triton X-100; pH 8.0), prepare the Lysozyme Buffer fresh immediately prior to use.*

1. Transfer bacterial culture (<10^9) to a microcentrifuge tube (not provided). Centrifuge for 1 min at 10,000 x g (13,000 rpm) and discard the supernatant.
2. Add 200µl of Lysozyme Buffer to the tube and vortex or pipette to resuspend the cell pellet.
   Incubate at room temperature for 10 minutes. During incubation, invert tube every 2-3 minutes.

3. Add 200µl GB Buffer to the sample. Vortex for 5 seconds to mix sample.
4. Incubate at 70°C for 10 minutes until the sample lysate is clear.
   During incubation, invert the tube every 3 minutes.

At this time, preheat required Elution Buffer (200µl per sample) at 70 °C.DNA Elution.

5. proceed to **DNA Binding Step (page 10).**

Yeast Protocol

Additional requirements:

Sorbitol Buffer, Lyticase or Zymolase, Microcentrifuge tube, Ethanol (96-100%), RNase A (10mg/ml)

Prepare Sorbitol Buffer:

1.2 M sorbitol; 10mM CaCl2 ; 0.1M Tris-Cl pH 7.5, 35mM Beta-mercaptoethanol

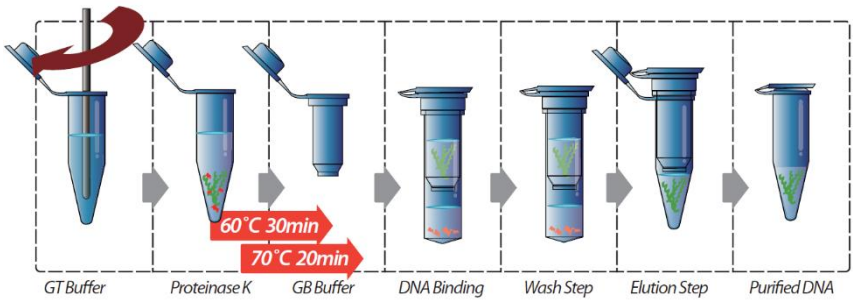
1. Harvest yeast cells (up to $5x10^7$) by centrifugation for 10 minutes at 5000 x g. Discard the supernatant and resuspend the cell pellet in 600µl sorbitol buffer.
2. Add 200U Lyticase or Zymolase. Incubate at 30°C for 30 minutes. Centrifuge the mixture for 10 min at 2,000 x g to harvest Spheroplast.
3. Remove the supernatant and add 200µl of GT Buffer to the tube and vortex or pipette to resuspend the cell pellet. Incubate at room temperature for 5 minutes.
4. Add 200µl GB Buffer to the sample. Vortex for 5 seconds to mix sample.
5. Incubate at 70°C for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

At this time, preheat required Elution Buffer (200µl per sample) at 70°C.DNA Binding.

6. proceed to **DNA Binding Step (page 10).**

Tissue Protocol

OverView



Add 1.1ml ddH_2O to a Proteinase K (11mg) tube, vortex to dissolve. Store prepared Proteinase K at 4 ˚C.
Add 100ml ethanol (96-100%) to Wash Buffer before initial use.
Additional Requirement: *Microcentrifuge tube, Ethanol (96-100%), RNase A (10mg/ml)*

Tissue Dissociation

1. Cut up 20mg of animal tissue (or 0.5cm of mouse tail) and transfer to a microcentrifuge tube (not provided).If tissue has a higher number of cells (e.g. spleen or liver), reduce starting material to 10mg.
2. Use provided micropestle to grind the tissue to a pulp.
3. Add 200µl GT Buffer into the tube and continue to homogenize the sample tissue with grinding.

Cell Lysis

4. Add 20µl Proteinase K (10mg/ml) to the sample mixture and vortex to mix. Incubate at 60˚C for 30 minutes to lyse the sample. During incubation, invert the tube every 5 min.
5. Add 200µl GB Buffer and vortex for 5 seconds to mix sample.
6. Incubate at 70˚C for 20 minutes until the sample lysate is clear. During incubation, invert the tube every 5 min.

At this time, preheat required Elution Buffer (200µl per sample) at 70 ˚C.DNA Elution.

**If there is insoluble material present following incubation, centrifuge for 2 minutes at full speed (13,000 rpm) and transfer the supernatant to a new microcentrifuge tube (not provided).*

7. proceed to **DNA Binding Step (page 10).**

For Paraffin-embedded Tissue Protocol

Additional Requirement: *Xylene, Microcentrifuge Tube, Ethanol (96-100%)*

Sample Preparation

1. Slice small sections (up to 25mg) from blocks of paraffin-embedded tissue and transfer to a microcentrifuge tube.
2. Add 1ml xylene to each tube. Vortex vigorously and incubate at room temperature for about 10 min. Vortex occasionally during incubation step.
3. Centrifuge at 10,000 x g (13,000 rpm) for 3 min. Remove supernatant by pipetting.
4. Add 1 ml ethanol to wash sample pellet and mix by inverting.
5. Centrifuge at 10,000 x g (13,000 rpm) for 3 min. Remove supernatant by pipetting. Repeat the Wash Step.
6. Open tube and incubate at 37°C for 15 minutes to evaporate the residual ethanol.
7. Add 200µl GT Buffer to suspend the sample
8. Proceed to **Cell Lysis Step 4 of Tissue protocol (page 8).**

For Buccal Swab Protocol

Additional Requirement:
Swab: cotton, DACRON or C.E.P. swabs; PBS; Microcentrifuge Tube; Ethanol (96-100%)

Sample Preparation

1. Scrape the swab firmly against the inside of each cheek 6-7 times and air dry the swab.(Sample donor should not ingest anything for at least 30 minutes prior to sample collection.)
2. Separate the swab cotton form the stick. Place the swab into a 2ml microcentrifuge tube, add 500µl PBS.
3. Add 500µl GB Buffer and 20ul Proteinase K (10mg/ml). Vortex for 5 seconds to mix sample.
4. incubate at 70°C for 20min.During incubation, invert the tube every 5 minutes.

At this time, preheat required Elution Buffer (200µl per sample) at 70 °C.DNA Elution.

5. Add 500µl of ethanol (96-100%) to the sample lysate and vortex immediately to mix.
6. Place a GD Column on a 2 ml Collection Tube.
7. Apply 700µl of the mixture from the previous step to the GD Column.
8. Close the cap and centrifuge at 10,000 x g (13,000 rpm) for 1 minute. Discard the flow-through and return the GD column to the 2ml Collection Tube
9. Repeat Step 5-8 by applying the remaining mixture to GD Column.
10. proceed to **Wash Step (page 10).**

Optional Step: RNA Degradation

If RNA-free genomic DNA is required, perform this optional step.

a.Add 5µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix.

b.Incubate at room temperature for 5 minutes.

DNA Binding

1. Add 200µl of ethanol (96~100%) to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting.
2. Place a GD Column on a 2ml Collection Tube.
3. Apply the total mixture (including any precipitate) from previous step to the GD Column.
4. Close the cap and centrifuge at 10,000 x g (13,000 rpm) for 5 minutes.
5. Discard the flow-through and return the GD column to the 2ml Collection Tube.

Wash

1. Add 400µl of W1 Buffer to the GD Column. Centrifuge at 10,000 x g (13,000 rpm) for 30 seconds.
2. Discard the flow-through and return the GD Column to the 2ml Collection Tube.
3. Add 600µl of Wash Buffer (ethanol added) in the GD Column Centrifuge at 10,000 x g (13,000 rpm) for 30 seconds.
4. Discard the flow-through and return the GD Column to the 2ml Collection Tube, Centrifuge at 10,000 x g (13,000 rpm) for 3 minutes to dry the column matrix.

Elution

DNA Elution

Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30-50µl) to increase DNA concentration.
If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to about 200µl.

1. Transfer dried GD Column into a clean 1.5 ml microcentrifuge tube (not provided).
2. Add 100µl of preheated Elution Buffer to the centre of the column matrix.
3. Allow to stand for 3-5 minutes until Elution Buffer is absorbed by the matrix.
4. Centrifuge at 10,000 x g (13,000 rpm) for 30 seconds to elute purified DNA.

Content and Storage

Genomic DNA Extraction Kit Mini

Plant

Cat.No. YGP50//YGP100

Kit Contents

Cat.No. YGP50
50 mini preps / kit

GP1 Buffer.....25 ml
GPX1 Buffer.....25 ml
GP2 Buffer.....6 ml
GP3 Buffer*.....15 ml
W1 Buffer.....25 ml
Wash Buffer (Concentrated)**...25 ml
Elution Buffer.....30 ml
RNase A (10mg/ml).....275 µl
Filter Column.....50 pcs
GD Column.....50 pcs
2 ml Collection Tube.....100 pcs

Cat.No. YGP100
100 mini preps / kit

GP1 Buffer.....50 ml
GPX1 Buffer.....50 ml
GP2 Buffer.....15 ml
GP3 Buffer*.....30 ml
W1 Buffer.....50 ml
Wash Buffer (Concentrated)**...25 ml
Elution Buffer.....30 ml
RNase A (10mg/ml).....550 µl
Filter Column.....100 pcs
GD Column.....100 pcs
2 ml Collection Tube.....200 pcs

Sample (Protocols Included): *1g of plant tissue* ***Yield:*** *300 µg* ***Operation time:*** *< 60 mins*
Elution Volume: *50µl*

* *Add 2 times volume isopropanol to GP3 Buffer prior to initial use.*
** *Add 4 times volume ethanol (96-100%) to Wash Buffer prior to initial use.*
Additional Requirement : *15ml , 50ml Centrifuge Tube.*

Description

The Plant Genomic DNA Extraction Kit (Duo Buffer System) provides a fast and simple method to isolate DNA from plant tissue and cells. In the first step, the sample is lysed by homogenization. The lysate is treated with RNase A to remove RNA. In the presence of a chaotropic salt, the genomic DNA in the lysate binds to the glass fiber matrix in the spin column. The contaminants are washed with an ethanol based wash buffer and purified genomic DNA is eluted by low salt elution buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 minutes.

Quality Control

The quality of Genomic DNA Extraction Mini Kit (Tissue) is tested on a lot-to-lot basis. The kits are tested by isolation of genomic DNA from 10mg of mouse liver. Purified DNA is quantified with a spectrophotometer. Genomic DNA yield is more than 10µg with A260/A280 ratio 1.7 to 1.9 .

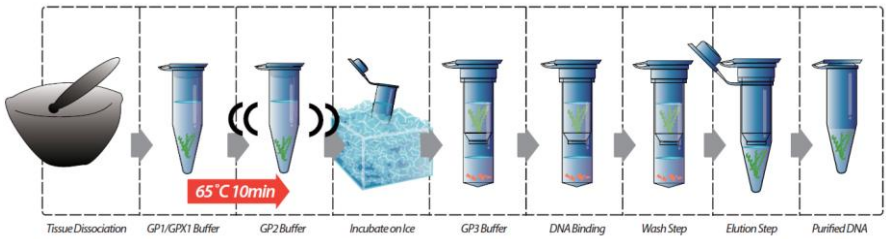
Reference

Vogelstein, B., and Gillespie , D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Note

* For research use only. Not for use in diagnostic or therapeutic procedures.
* Buffer contains guanidine hydrochloride which is harmful and can act as an irritant. During operation, always wear a lab coat, disposable gloves and protective goggles.

Overview Protocol



Additional Requirements:

Liquid Nitrogen , Mortar, 1.5 ml microcentrifuge tube, Ethanol (96-100%), Isopropanol.

Protocol Modification (Duo Buffer System)

Plant species are extremely diverse in their metabolic components. Large amounts of polysaccharides, carbohydrates, lipids, poly-phenols and proteins may be distributed throughout the plant tissue. These compounds often interfere with DNA binding and extraction. Due to this characteristic of plants, we offer two lysis buffers for optimum performance according to different plant samples.

GP1:

The standard protocol utilizes **GP1 buffer** for plant sample lysis. This buffer system is suitable for most common plant species, to ensure high quality and high yield of DNA.

GPX1:

An alternative buffer, **GPX1 buffer**, is also included with this kit. The detergent present in this buffer is more effective in dispersing plant samples with large amounts of polysaccharide. For most of plant species, both buffers give similar results. Researchers may try one buffer system first or both in parallel.

Tissue Dissociation

1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue or 5 mg (up to 100 mg) of dried sample.
2. Grind the sample with mortar and pestle under liquid nitrogen to a fine powder. For some plant samples, liquid nitrogen may be unnecessary for homogenization.
3. Transfer it into a microcentrifuge tube (not provided).

Lysis

4. Add 400 µl GP1 Buffer (or GPX1) and 5 µl RNase A (10mg/ml) into the sample tube and mix by vortexing. Do not mix GP1 (GPX1) Buffer with RNase A before use.
5. Incubate at 65°C for 10 minutes. During incubation, invert the tube every 5 minutes. At the same time, preheat required Elution Buffer (200 µl per sample) at 65°C.
6. Add 100µl GP2 Buffer and mix by vortexing.
7. Incubate on ice for 3 minutes. Place a Filter Column into a 2 ml Collection Tube and apply the entire lysate from previous step to the Filter Column.
8. Centrifuge for 3 minutes at full speed (13,000 rpm).
9. Discard the Filter Column and carefully transfer clarified supernatant in the Collection Tube to a new microcentrifuge tube (not provided).

DNA Binding

10. Add 1.5 times volume of GP3 Buffer (isopropanol added) to the cleared lysate and mix immediately by vortexing for 5 seconds. For example, add 750 µl GP3 Buffer to 500 µl lysate.
11. Place a GD Column into a 2 ml Collection Tube.
12. Apply 700 µl of the mixture (including any precipitation) from previous step to the GD Column.
13. Centrifuge at full speed (13,000 rpm) for 2 minutes.
14. Discard flow-through in Collection Tube and apply remaining mixture to GD Column.
15. Repeat step12-14 again. Apply the remaining mixture to the same GD Column

Wash

17. Add 400 µl of W1 Buffer to the GD Column.
18. Centrifuge at full speed (13,000 rpm) for 30 seconds.
19. Discard the flow-through and place the GD Column back in the Collection Tube.
20. Add 600 µl of Wash Buffer (ethanol added) to the GD Column.
21. Centrifuge at full speed (13,000 rpm) for 30 seconds
22. Discard the flow-through and place the GD Column back in the Collection Tube
23. Centrifuge at full speed for 3 minutes to dry out column matrix.

Optional Step: RNA Degradation

If a few pigments remain on the column matrix, perform this procedure.

a. After Wash Steps are completed, add 400 µl of ethanol (96-100%) in the GD Column.

b. Centrifuge at full speed (13,000 rpm) for 30 seconds

c. Discard the flow-through and place the GD Column back in the Collection Tube

d. Centrifuge at full speed for 3 minutes to dry out column matrix.Following 70 ̈C incubation, add 5µl of RNase A (10mg/ml) (not provided) to sample lysate and vortex to mix.

e. Incubate at room temperature for 5 minutes.

DNA Elution

Standard elution volume is 100µl. If less sample to be used, reduce the elution volume (30-50µl) to increase DNA concentration.

If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to about 200µl.

24. Transfer dried GD Column to a clean 1.5 ml microcentrifuge tube (not provided)
25. Add 100 µl of preheated Elution Buffer to the center of the column matrix.
26. Allow to stand for 3-5 minutes until Elution Buffer is absorbed by the matrix.
27. Centrifuge at full speed (13,000 rpm) for 30 seconds to elute purified DNA.

Troubleshooting

Column clogged	***Overloaded column with sample*** *Reduce sample volume or separate into multiple tubes.*
	Precipitate was formed at DNA Binding Step *Reduce the sample material.* *Prior to loading the column, break up precipitate in ethanol-added lysate.*
Low yield	***Incorrect DNA Elution Step*** *Ensure that Elution Buffer was added and absorbed to the centre of GD Column matrix.*
	Incomplete DNA Elution *Elute twice to increase yield*
Eluted DNA does not perform well in downstream applications	***Residual ethanol contamination*** *Following the wash step, dry GD Column with additional centrifugation at full speed for 5 minutes or incubation at 60℃ for 5 minutes.*
	RNA Contamination *Perform optional RNA degradation step.*
	Protein Contamination *Reduce the sample amount After DNA Binding Step, apply 400 µl W1 Buffer to wash GD Column and centrifuge at 13,000rpm for 30 seconds. Proceed with Wash Step*
	Genomic DNA was degraded *Use fresh tissue sample; prolonged storage may result in fragmentation of genomic DNA.*