DATA SHEET

Geno *Plus*™ Genomic DNA Extraction Miniprep System

Viogene Geno *Plus*™ Genomic DNA Extraction System provides a simple and fast way to purify genomic DNA (including viral or mitochondrial DNA) from various sources such as whole blood (anticoagulant added), animal tissue, mouse tail, serum, buffy coat, cultured animal cells, bacteria, yeast, virus, lymphocytes, paraffin-embedded tissue, plasma and body fluids. A simple spin column procedure can purify pure DNA (approximately 20-30 kb fragment) for PCR, enzymatic reactions, and other downstream applications. 0.3 ml whole blood volume will yield up to 15 µg of genomic DNA.

Downstream Application

* Restriction digestion
* Southern Blotting
* RAPD, RFLP
* PCR, Real-Time PCR

Product Contents

Cat. No	GG2001	GG2002
Preps	50	250
RL Buffer	100ml	250ml X 2
LYS Buffer	12ml	60ml
FX Buffer	17ml	85ml
WS Buffer	15ml	45ml X 2
Proteinase K	10mg	10mg X 5
B/T Genomic DNA Mini Column	50	250
Collection Tube	100	500
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Geno *Plus*™ Genomic DNA Extraction Miniprep System is shipping and storage at ambient temperature up to 12 months.

If precipitate form by freezing temperature on any buffer, warm up at 37℃ to redissolve.

Protocol

❖ **Please read the following notes before starting the procedures.**

Important Notes

- RNA may be copurified with genomic DNA. RNA will not affect PCR, but may affect certain downstream applications. If RNA-free genomic DNA is desired, add RNase A to the sample as indicated in the protocol.
- DNA can be eluted in 10 mM Tris-HCl (pH 9.0), Milli-Q, double-distilled H_2O, or TE buffer (pH 8.0). Since genomic DNA elution takes place most effectively at pH 9, to ensure optimal elution, make sure that pH of these elution solution are between 8.0 and 9.0.
- Add 60 (for GG2001) or 180 (for GG2002) ml of 98-100% ethanol into WS Buffer bottle when first open.
- Add 1 ml sterile ddH_2O to reconstitute the provided Proteinase K by vortexing. Store the solution at 4 ℃.
- Buffers in this system contain irritants. Appropriate safety apparels such as gloves and the lab coat should be worn to protect from skin contact.
- All procedure should be done at room temperature (20-25 ℃).
- Centrifuge steps done at full speed refers to 10,000 x g or 13,000-14,000 rpm of a microcentrifuge, if no notice.

I. Blood Protocol: (Refer to Page 3 for Nucleated Blood Cell Samples)

1. **Pipet up to 300 μl whole blood (anticoagulant added) into a 1.5-ml sterile eppendorf tube. Add 1.0 ml RL Buffer, mix well by invert, and incubate for at least 5 minutes to lyse RBC.**

2. **Centrifuge at 3,000 x g (5000 rpm) for 3 minutes, then discard the supernatant.**

3. **Add 0.8 ml RL Buffer and invert several times to mix well. Centrifuge at t 3,000 x g (5000 rpm) for 2 minutes, then discard the supernatant.**

4. **Add 20 μl Proteinase K and 180 μl LYS Buffer into the sample. Mix immediately by vortexing for 20 seconds.**
   Do not add Proteinase K directly to LYS Buffer.

5. **Incubate at 60 ℃ for 30 minutes to lyse the sample. Vortex or invert the sample every 3-5 minutes during incubation.**
   Ensure complete sample lysis: whole blood sample should NOT appear viscous; buffy coat should NOT contain insoluble residues; cell sample should appear translucent.
   If RNA-free genomic DNA is desired, add 10 μl of 50 mg/ml RNase A to the sample and mix well after 60℃ incubation, and incubate for 10 minute or more at room temperature.

6. **Add 300 μl FX Buffer into the sample, and mix well by invert.**

7. **Preheat 10 mM Tris-HCl (pH 9.0), ddH$_2$O, or TE buffer (provided by user) at 70 ℃ (500 μl/prep) for DNA elution.**

8. **Add 200 μl of ethanol (98-100%) or isopropanol to the sample of Step 6 and mix well by invert.**

9. **Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipet all the mixture (including any precipitate) into the column without touching the rim.**

10. **Centrifuge at 8,000 rpm (6,000 x g) for 2 minutes. Place the column onto a new Collection Tube.**

11. **Wash the column twice with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x g) for 2 minutes. Discard the flow-through.**
    Ethanol (98-100%) must be added when first open the WS Buffer bottle.

12. **Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.**

13. **Place the column onto a new 1.5-ml tube (provided by user). Elute DNA with 200 μl of the preheated elution solution from Step 7.**

14. **Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.**

15. **Store eluted DNA at 4 ℃ or –20 ℃.**
    Store DNA at 4 ℃ for frequent use or at –20 ℃ for long-term storage. Repeated freeze-thaw cycles can cause shearing of genomic DNA.

* Direct Blood Protocol:

For the sample of nucleated blood cell, such as bird or fish.

1. **Apply up to 20 µl of nucleated blood cell sample into a 1.5-ml sterile eppendorf tube, add 180 µl LYS Buffer and mix well. And then follow the Step 2 of Tissue Protocol on this page.**

II. Tissue Protocol:

1. **Cut 30 mg of tissue (15 mg spleen) into small pieces and place the sample into a 1.5-ml sterile eppendorf tube. Add 180 µl LYS Buffer and homogenize the sample.**

   If the sample size is larger than 30 mg, increase the amount of LYS Buffer proportionally.

2. **Add 20 µl Proteinase K to the sample. Mix immediately by vortexing for 20 seconds.**

3. **Incubate at 60 ℃ for 1 hour to lyse the sample. If tissue is difficult to lyse, increase the incubation time to 2-3 hours. Vortex or invert the sample every 10-15 minutes.**

   Ensure complete sample lysis; sample after complete lysis should appear translucent.

4. **Add 300 µl of FX Buffer to the sample, and mix by vortexing. Adjust the incubator to 70 ℃ to incubate for 20 minutes.**

   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample and mix well after 70℃ incubation, and incubate for 10 minute or more at room temperature.

5. **Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70 ℃ (500 µl/prep) for DNA elution.**

6. **Add 200 µl of ethanol (98-100%) or isopropanol to the sample and mix by vortexing.**

   If the sample mixture is more than 550 µl, increase the amount of ethanol proportionally.

7. **Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipet all the mixture (including any precipitate) into the column without touching the rim. Centrifuge at 8,000 rpm (6,000 x g) for 2 minutes. Place the column onto a new Collection Tube.**

   If a precipitate formed from step 6, apply the precipitate and mixture to the B/T Genomic DNA Mini Column.

   If the B/T Genomic DNA Mini Column clogged after 2 minutes spin, centrifuge again at full speed for another 2 minutes.

8. **Wash the column twice with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x g) for 2 minutes. Discard the flow-through.**

   Ethanol (98-100%) must be added when first open the WS Buffer bottle.

9. **Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.**

10. **Place the column onto a new 1.5-ml tube (provided by user). Elute DNA with 200 µl of the preheated elution solution from Step 5.**

11. **Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.**

12. **Store eluted DNA at 4 ℃ or −20 ℃.**

III. Mouse Tail protocol:

1. **Cut into small pieces of a segment of mouse tail of up to 0.5 cm. Place the sample into a 1.5-ml sterile tube.**
   Segment close to the tail tip is preferred. Segment away from the tip is thicker and takes longer time to lyse completely.

2. **Add 20 µl Proteinase K and 180 µl LYS Buffer to the sample. Mix immediately by vortexing for 20 seconds.**
   Further addition of 20 µl of 10 mg/ml Proteinase E (DNase-free, provided by user) can enhance mouse tail lysis and increase DNA yield.

3. **Incubate at 60 ℃ for 1-4 hours or overnight to lyse the tail tissue. Vortex or invert the sample every 20-30 minutes during incubation.**
   Ensure complete sample lysis; sample after complete lysis should appear translucent with only hair and bone residues remained.

4. **Use the supernatant only and follow the Tissue Protocol starting from Step 4 on Page 3.**

IV. Serum protocol:

For sample including buffy coat, plasma, body fluid, Lymphocytes and cultured animal cells in 200 µl of PBS. Sample volume should not be larger than 200 µl.

1. **Add 20 µl Proteinase K and 150 µl FX Buffer to the sample. Mix immediately by vortexing for 20 seconds.**

2. **Incubate at 60 ℃ for 40 minute to lyse the cells. Vortex or invert the sample every 5 minutes during incubation.**
   Incubation with mixing facilitates lysis. Ensure complete cell lysis; sample after complete lysis should appear translucent.

3. **Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70 ℃ (500 µl/prep) for DNA elution.**

4. **Add 150 µl FX Buffer into the sample and mix well by invert.**

5. **Follow the Tissue Protocol starting from Step 6 on Page 3.**

V. Paraffin-Embedded Tissue Protocol

1. **Cut a small section of paraffin-embedded tissue (about 25 mg) and put into a 1.5-ml eppendorf tube.**

2. **Add 1 ml xylene and incubate at room temperature with occasional mixing for 30 minutes to extract paraffin from tissue.**

3. **Centrifuge at full speed for 5 minutes. Remove the supernatant by pipetting.**

4. **Add 1 ml absolute ethanol to the tissue pellet, mix, and centrifuge at full speed for 5 minutes. Remove ethanol-containing xylene residue by pipetting.**

5. **Evaporate ethanol residue by incubating at 37 ℃ for 10 minutes.**

6. **Resuspend the pellet in 180 µl LYS Buffer.**

7. Follow the Tissue Protocol starting from Step 2 on Page 3.

VI. Bacteria Protocol

A. For Gram-positive and Gram-negative bacteria

1. Pellet log-phase grown bacteria of up to 10^9 (or up to 3 ml culture) at 7,500 rpm (5,000 x g) for 10 minutes.

2. Completely resuspend the pellet in 160 µl TE buffer, then add 40 µl of lysozyme solution (100mg/ml) and mix well.

3. Incubate at 37 ℃ for 30 minutes.

4. Add 20 µl Proteinase K and 150 µl FX Buffer to the sample. Mix immediately by vortexing for 20 seconds.

5. Incubate at 60 ℃ for 40 minute to lyse the bacterial cells. Vortex or invert the sample every 5 minutes during incubation.
Incubation with mixing facilitates lysis. Ensure complete cell lysis; sample after complete lysis should appear translucent.
If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample and mix well after 60℃ incubation, and incubate for 10 minute or more at room temperature.

6. Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70 ℃ (500 µl/prep) for DNA elution.

7. Add 150 µl FX Buffer into the sample and mix well by invert.

8. Follow the Tissue Protocol starting from Step 6 on Page 3.

B. For bacteria in biological fluids

1. Pellet cells by centrifuging at 7,500 rpm (5,000 x g) for 10 minutes.

2. Resuspend the pellet in 180 µl LYS Buffer.

3. Follow the Tissue Protocol starting from Step 2 on Page 3.

C. For bacteria from eye, nasal, or pharyngeal swabs

1. Collect bacterial cells by rinsing and soaking the swabs in 2 ml PBS at room temperature for 2-3 hours.

2. Pellet cells by centrifuging at 7,500 rpm (5,000 x g) for 10 minutes.

3. Resuspend the pellet in 180 µl LYS Buffer.

4. Follow the Tissue Protocol starting from Step 2 on Page 3.

VII. Yeast Protocol

1. **Pellet log-phase grown yeast cells up to 10^8 (or up to 3 ml culture) at 7,500 rpm (5,000 x g) for 10 minutes.**

2. **Resuspend the pellet in 0.5 ml sorbitol reaction solution (1 M sorbitol; 100 mM EDTA; 14 mM β-mercaptoethanol; 200 U lyticase or zymolase).**

3. **Incubate at 30℃ for 30 minutes.**

4. **Centrifuge at 7,500 rpm (5,000 x g) for 5 minutes. Resuspend the pellet in 180 µl LYS Buffer.**

5. **Follow the Tissue Protocol starting from Step 2 on Page 3.**

VIII. Virus Protocol

1. Prepare viral DNA from blood or body fluid, the **Serum Protocol** is recommended.

2. Prepare integrated viral DNA, the **Tissue Protocol** is recommended.

Troubleshooting

1. Brown color residues remain on the membrane of Genomic DNA column after washing

a. Incomplete digestion of Hemoglobin

Vortex the sample after Proteinase K is added. Mix the sample every 3-5 minutes during incubation.

b. No alcohol added to the sample before loading onto the Genomic DNA column

Before passing the column, add more than 200 µl (or suitable volume) of absolute ethanol or isopropanol to the sample.

c. Incorrect amount of ethanol added to the WS Buffer

Make sure that ethanol is added into the WS Buffer bottle when first open.

2. Little or no DNA in the elute

a. Sample contains too low amount of genomic DNA

Increase the sample amount, Proteinase K, and buffer proportionally. If the sample is whole blood, prepare buffy coat from a larger volume of blood.

b. Blood sample is not lysed completely

Add another 20 µl fresh Proteinase K per sample and repeat incubation.

c. No alcohol added to the sample before loading onto the Genomic DNA column

Before passing the column, add more than 200 µl (or suitable volume) of absolute ethanol or isopropanol to the sample.

d. Incorrect amount of ethanol added to the WS Buffer

Make sure that ethanol is added into the WS Buffer bottle when first open.

e. Elution solution is not preheated at 70 ℃

Preheat the elution solution at 70 ℃ before used.

f. The pH value of the elution solution is too low

Make sure that the pH value of 10 mM Tris-HCl, ddH_2O or TE buffer for elution is between 8.0-9.0.

3. Column is clogged when passing the sample

a. Tissue sample contains undigested remains

After Proteinase K digestion, centrifuge the sample at full speed for 5 minutes to remove undigested remains.

b. Blood sample contains clots

Use whole blood sample mixed well with anticoagulant to prevent formation of blood clot.

Do not use blood clot for genomic DNA extraction.

c. Sample is very viscous

Too much sample is used. Reduce the sample amount.

4. A_{260}/A_{280} ratio of eluted genomic DNA is low

a. Protein in the sample is not completely degraded

Vortex the sample after Proteinase K is added. Mix the sample at constant intervals during incubation. Add 20 µl fresh Proteinase K per sample and continue incubation.

b. No alcohol added to the sample before loading onto the Genomic DNA column

Before passing the column, add more than 200 µl (or suitable volume) of absolute ethanol or isopropanol to the sample.

c. Eluted genomic DNA contains contaminants.

Do not touch the rim of the column during sample or buffer loading.

d. Eluted genomic DNA contains ethanol

After the final wash, centrifuge the column at full speed for another 2 minutes to remove the ethanol residue completely.

e. Using ddH_2O of acidic pH (5.0-6.0) to dilute DNA samples for spectrophotometric analysis

Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute the DNA samples.

5. A_{260}/A_{280} ratio for genomic DNA is high (over 1.9)

a. RNA contamination

Add RNase A to the sample as described in the protocol.

6. Genomic DNA appears smearing and degraded

a. Sample is not fresh or stored improperly for a long time

Flash freeze fresh samples in liquid nitrogen and store at −80 ℃ if not used immediately.

b. Blood sample is not fresh or stored improperly for a long time

Use fresh blood, or blood stored at room temperature for fewer than 2 days.

c. Gel electrophoresis is performed in used running buffer contaminated with DNase

Use fresh TAE or TBE running buffer for electrophoresis.

d. Paraffin-embedded tissue is used as sample

Genomic DNA isolated from this kind of sample is usually degraded. It is still suitable for PCR application, but is not recommended for Southern blotting and restriction analysis.