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VIOGENE
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User Bulletin

Geno *Plus* Genomic DNA Extraction Maxiprep System

Viogene Geno Plus Genomic DNA Extraction Maxiprep System provides a simple and fast method to purify genomic DNA (including viral or mitochondrial DNA) from whole blood (anticoagulant added). A simple spin column procedure can purify genomic DNA (approximately 20-30 kb fragment) for PCR, enzymatic reactions, and other downstream applications. 10 ml whole blood volume will yield 100-200 μg of genomic DNA.

Downstream Application

* Restriction digestion
* Sequencing
* RAPD, RFLP
* PCR, Real-Time PCR

Product Contents

Cat. No	GGM2001	GGM2002
Preps	10	25
RL Buffer	265ml X 2	265ml X 5
LYS Buffer	20ml	50ml
FX Buffer	35ml	85ml
WS Buffer	50ml	50ml X 3
Proteinase K	15mg X2	15mg X 4
B/T Genomic DNA Maxi Column	10	25
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Geno *Plus* Genomic DNA Extraction Maxiprep System can be shipped and stored at ambient temperature up to 12 months.

If precipitate form by cold temperature on any buffer, warm the buffer 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 elutes 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 200 ml of 98-100% ethanol into WS buffer bottle when first open.
- Add 1.5 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 lab coat should be worn to protect from direct contact.
- All procedure should be done at room temperature (20-25 ℃).

1. **Pipet 8 ml (up to 10 ml) whole blood (anticoagulant added) into a 50ml sterile tube. Add 30 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 another 20 ml RL Buffer and invert several times to mix well, and stand at RT for 5 min, Centrifuge at 3,000 x g (5000 rpm) for 2 minutes, then discard the supernatant.**

4. **Add 200 µl Proteinase K and 1.8 ml 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 1 hour 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; the sample should appear translucent.

6. **Adjust the incubator to 70 ℃ and incubate for 10 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 minutes at room temperature.

7. **Add 3 ml FX Buffer into the sample, and mix well by inverting.**

8. **Preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70 ℃ for DNA elution.**

8. **Add 2 ml of ethanol (98-100%) or isopropanol to the sample of Step 7 and mix well by inverting.**

9. **Place a B/T Genomic DNA Maxi Column onto a 50 ml centrifugation Tube. Pipet all the mixture (including any precipitate) into the column without touching the rim.**

10. **Centrifuge at 3,000 rpm (5,000 x g) for 3 minutes. Place the column onto a new Collection Tube.**

11. **Wash the column twice with 12 ml WS Buffer by centrifuging at 3,000 rpm (5,000 x g) for 3 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 4,000 rpm (6,000 x g) for another 10 minutes to remove ethanol residue.**

13. **Place the column onto a new 50 ml tube (not provided). Elute DNA twice with 1 ml each of the preheated elution solution from Step 8.**

14. **Stand the column for 5 minutes before centrifugation, and centrifuge at full speed for 2-5 minutes to elute DNA. (Optional the third elution with 1 ml elution solution will yield extra DNA, is also recommended).**

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 genomic DNA shearing.

Troubleshooting

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

a. Incomplete digestion of Hemoglobin, or too much (>10 ml) blood sample used

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 2 ml 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 200 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 2 ml 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 with well mixed 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 digested and degraded

Vortex the sample after Proteinase K is added. Mix the sample at constant intervals during incubation. Add 200 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 2 ml 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

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 reused 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.