

VIogene

User Bulletin

Cat. #: GGD1002

Blood Genomic DNA Extraction Midiprep System

Viogene Blood Genomic DNA Extraction Midiprep System provides a simple and fast way to purify total genomic DNA (including viral or mitochondrial DNA) from various sources such as blood, plasma, serum, buffy coat, lymphocytes and body fluids. A simple spin column procedure can purify pure DNA (approximately 20-30 kb fragment) for PCR, enzymatic reactions, and other downstream application. 1 ml whole blood volume will yield 10-50 µg of genomic DNA.

Kit Contents:

EX Buffer (1), WS Buffer (3), Proteinase K powder (1), Genomic DNA Midi Column (100), Collection tube (100) and protocol (1)

Notes:

- All procedures should be done at room temperature.
- Prepare a 60°C (and an optional 70°C) water bath.
- Add 180 ml of 98% ethanol to WS Buffer bottle when first Open.
- RNA may also copurify with genomic DNA, copurified RNA will not inhibit PCR reaction, but may inhibit some downstream enzymatic reactions. If RNA-free genomic DNA is required, add 50 µl of 50 mg/ml RNase A (DNase free) to the sample.

Protocol:

- 1. Pipette 1 ml sample into a 15 ml tube.**
Samples: Whole blood, plasma, serum, buffy coat, body fluids, or 10⁷-10⁸ lymphocytes in 1 ml PBS.
If the sample volume is less than 1 ml, add the appropriate volume of PBS to make up 1 ml.
- 2. Add 1.2 ml ddH₂O to the Proteinase K powder tube (provided) and vortex for 1 minute to completely dissolve Proteinase K.**
The completely dissolved Proteinase K should look transparent, if the tube looks turbid, keep vortex until complete resolution of Proteinase K. The concentration of dissolved Proteinase K is 25 mg/ml.
- 3. Add 10 µl Proteinase K and 1 ml EX Buffer to the sample. Mix immediately by vortexing for 20 seconds.**
If sample volume is larger than 1 ml, increase the amount of EX Buffer and Proteinase K proportionally. Do not add Proteinase K directly to EX Buffer and store dissolved Proteinase K at 4 °C.
- 4. Incubate at 60°C for 20 minutes to lyse the sample, then turn the incubator to 70°C and incubate 20 minutes. Vortex or invert mix the sample every 3~5 minutes during incubation.**
Alternatively, place the sample to another 70°C incubator and incubate for 20 minutes.
Sample after complete lysis should not contain insoluble residues and appear viscous.
- 5. Preheat ddH₂O or 10 mM Tris-HCl, pH9.0 to 70°C (2.5 ml /prep) for DNA elution.**

- 6. Add 1,050 µl of isopropanol or ethanol (96-100%) to the 70°C-incubated sample of step 4 and mix by vortexing.**

If the sample volume is larger than 1 ml, increase the amount of isopropanol or ethanol proportionally.

- 7. Place a Genomic DNA Midi Column in a 15 ml Collection Tube (provided). Apply all the mixture from step 6 to the Genomic DNA Midi Column, and centrifuge at 2,500 x g (3,000 rpm for a swing-bucket centrifuge) for 3 minutes. Decant the filtrate in the 15 ml tube, and place the Genomic DNA Midi Column back to the tube.**

If a precipitate formed from step 6, apply the precipitate and mixture to the Genomic DNA Midi Column.

If Genomic DNA Midi Column clogged after 3 minutes spin, centrifuge again at full speed for another 3 minutes.

- 8. Add 2.5 ml of WS Buffer. Centrifuge at 2,500 x g (3,000 rpm for a swing-bucket centrifuge) for 3 minutes, and discard the filtrate.**

Add 180 ml of ethanol (96-100%) when first open the WS Buffer bottle.

- 9. Add another 2.5 ml of WS Buffer. Centrifuge at 2,500 x g (3,000 rpm for a swing-bucket centrifuge) for 3 minutes, discard the filtrate, and at full speed (about 4,000 rpm) for a further 5 minutes to dry the column.**

- 10. Place the Genomic DNA Midi Column in a new 15 ml tube (provided by user), and discard the Collection tube contains the filtrate.**

- 11. Elute the DNA with 1 ml of preheated ddH₂O or 10 mM Tris-HCl, pH 9.0 from step 5. Centrifuge at 2,500 x g (3,000 rpm for a swing-bucket centrifuge) for 10 minutes.**

Incubate the 1 ml ddH₂O or TE loaded column-tube 5 minutes at 70°C will increase DNA yield.

- 12. Store eluted DNA at -20°C.**

EDTA in TE elution buffer may inhibit PCR reaction, use ddH₂O elution for PCR is recommended.

Troubleshooting:

- 1. Brown color residues remain on the membrane of Genomic DNA Midi Column after washing**

- a. Incomplete digestion of Hemoglobin by Proteinase K.**

Prepare a new sample, add 20 µl (double amount) of Proteinase K stock (25 mg/ml) to 1 ml EX Buffer and vortex thoroughly, then incubate for 1 hour at 60°C to completely digest Hemoglobin.

- b. No alcohol added to the sample before loading onto the Genomic DNA Midi Column.**

Repeat the procedure with a new sample.

- c. Incorrect amount of ethanol added to the WS Buffer.**

- 2. Little or no DNA in the elute**

- a. Too low concentration of sample used.**

Increase the sample volume and repeatedly load into the Genomic DNA Midi Column.

- b. Incomplete cell lysis due to insufficient mixing of the sample with EX Buffer.**

Thoroughly vortex the sample with EX Buffer.

- c. **No alcohol added to the sample before loading onto the Genomic DNA Midi Column.**

Repeat the procedure with a new sample.

- d. **Elution buffer (ddH₂O or 10 mM Tris-HCl, pH 9.0) does not be heated to 70°C.**

Repeat elution with heated ddH₂O and incubate for 5 minutes at 70°C before spin.

- e. **The pH of Tris buffer is too low.**

The pH of 10 mM Tris-HCl must be 9.0.

- f. **Elute the DNA with less than 1 ml of elution buffer.**

Less than 1 ml of elution buffer will reduce yield.

3. A₂₆₀/A₂₈₀ ratio for genomic DNA is low

- a. **Inefficient cell lysis.**

Thoroughly vortex the mixture of sample.

- b. **Inefficient protein degradation.**

After adding Proteinase K, extend the 60°C incubation time.

- c. **No alcohol added to the sample before loading onto the Genomic DNA Midi Column.**

Repeat the procedure with a new sample.

- d. **Incorrect amount of ethanol added to the WS Buffer.**

4. A₂₆₀/A₂₈₀ ratio for genomic DNA is high (over 1.9)

- a. **RNA contamination.**

Use RNase A in step 3 of the protocol.