AuPreP Blood Genomic DNA Extraction Midi Kit

Cat. #: GBD51-185LT

AuPreP™ Blood Genomic DNA Extraction Midi Kit 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:

1. All procedures should be done at room temperature.
2. Prepare a 60°C (and an optional 70°C) water bath.
3. Add 180 ml of 98% ethanol to WS Buffer bottle when first Open.
4. RNA may also co-purify with genomic DNA, co-purified 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 10^7-10^8 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 ddH_2O 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 ddH_2O 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 ddH_2O 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 ddH_2O 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 ddH_2O 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 (ddH$_2$O or 10 mM Tris-HCl, pH 9.0) does not be heated to 70^{o}C.
Repeat elution with heated ddH$_2$O and incubate for 5 minutes at 70^{o}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. A$_{260/280}$ 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^{o}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. A$_{260/280}$ ratio for genomic DNA is high (over 1.9)

a. RNA contamination.
Use RNase A in step 3 of the protocol