PRODUCT INFORMATION

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EZ96-206 96-well Blood Genomic DNA miniprep kit:

Components	EZ96-206, 2 Plates	EZ96-206, 5 Plates
PBS Solution	40 ml	100 ml
Buffer CL	48 ml	120 ml
CW1 Solution (concentrated)	52 ml	2x65 ml
CW2 Solution (concentrated)	36	2x45
CE Buffer	40 ml	120 ml
Proteinase K	4.8 ml	12 ml
EZ96DBP plates (DNA Binding plates)	2	5
EZ96DWP plate (2ml deep well collection plates)	4	10
96 Storage plate	2	5
Sealing film	8	20
Protocol	1	1

(A) Before use, add 68 ml of 96-100% of ethanol to 52 ml of CW1 and 84 ml of 96-100% of ethanol to 36 ml of CW2.
Note: The purification method is based on centrifugation. Vacuum Manifold can also be used.

Principle

This 96-well blood genomic DNA miniprep kit is design for rapid and high-throughput purification of genomic DNA from fresh or frozen anticoagulated blood. Samples are first lysed using proteinase K in an optimized buffer. The lysate is then loaded onto a DNA binding plate (EZ96DBP). DNA is selectively bound to the silica-based membrane in each well of the plate in the presence of chaotropic salt. During wash steps, protein and other impurities are removed and the DNA is then eluted in water or low salt buffer.

Features:

- Fast, effective, and High quality of genomic DNA.
- Compatible with many downstream applications such as PCR, restriction enzyme digestions and hybridization.
- No phenol/chloroform extraction or ethanol precipitation.

Procedure:

1. Sample preparation.
   a) For non-nucleated erythrocyte blood (mammalian blood): pipet 20 µl proteinase K into each well on a deep well collection plate (EZ96DWP). Add 50-100 µl anticoagulated blood and adjust the volume to 220 µl with PBS. Continue with step 2.
   b) For nucleated erythrocyte blood (blood of birds, fish, frogs): Pipet 20 µl proteinase K into each well on a deep well collection plate (EZ96DWP). Add 5-10 µl anticoagulated blood and adjust the volume to 220 µl with PBS. Continue with step 2.
2. Add 200 µl Buffer CL to the sample and covered with sealing film, mix thoroughly by vortexing. Incubate at 56^{o}C for 10 min. NOTE: Add 20 µl RNaseA (20 mg/ml) if RNA-free genomic DNA is required. Mix well and incubate at room temperature for 2 min before continuing with step 3.
3. Add 200 µl ethanol (96-100%) and covered with a new sealing film, mix thoroughly by vortexing.
4. Transfer the mixture from step 3 (including precipitate) to a DNA binding plate (EZ96DBP). Place the binding plate on top of a new 96-deep-well collection plate. Centrifuge at 5,000xg (6,000 rpm) for 1 min. Discard the flow-through.
5. Add 500 µl CW1 Solution (with ethanol), and centrifuge for 1 min at 5,000xg (6,000 rpm). Discard the flow-through.
6. Add 500 µl CW2 Solution (with ethanol), and centrifuge for 1 min at 5,000xg (6,000 rpm). Discard the flow-through.
7. Place the DNA Binding plate in a 96-deep-well collection plate and centrifuge for an additional 2 min to dry the membrane in binding plate (residual ethanol may affect downstream applications). Transfer the binding plate to a 96-well storage plate.
8. Add 50-100 µl CE buffer directly onto the center part of membrane of each well in the binding plate. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 5,000 x g to elute DNA.
   NOTE: for better yield of genomic DNA, warm up CE buffer and add more CE buffer for 2nd elution.