Column-Pure Blood Genomic DNA Mini Kit

Cat. No. D203

Store all buffers at 18-25°C; Store Proteinase K at -20°C.

Product Description

abm's Column-Pure Blood Genomic DNA Mini Kit is a quick and easy silica spin column method for isolation of genomic DNA from whole blood samples. The purified DNA can be used in downstream applications such as PCR, RT-qPCR, NGS and sequencing.

Product Component	Quantity
PBS Solution	8 ml
Buffer CL	12 ml
CW1 Solution	13 ml
CW2 Solution	9 ml
CE Buffer	15 ml
Buffer TBP	50 ml
Proteinase K	1.2 ml
EZ-10 Spin Columns	50

Protocol

Before use, add 100% Ethanol to CW1 Solution (17 ml) and CW2 Solution (21 ml).
Perform all centrifugation steps at 10,000 rpm unless stated otherwise.

1. Prepare blood samples.
   1a. **Non-Nucleus Erythrocytes** (e.g. human blood): Collect ~100 µl of blood into a 2.0 ml centrifuge tube. Add PBS Solution to a final volume of 200 µl. Vortex gently and incubate at room temperature for 1 min. If >100 µl sample, add 2 volumes of Buffer TBP. Mix thoroughly and incubate at room temperature for 1 min until red cells lyse completely. Centrifuge at 8000 rpm for 1 min, discard the supernatant. Wash the precipitate with 500 µl TE Buffer (not included), centrifuge at 8000 rpm and discard the supernatant. Repeat. The final precipitate should appear white in color. Proceed to Step 2.

   1b. **Nucleus-containing Erythrocytes** (e.g. chicken blood): Collect ~10 µl of blood into a 2.0 ml centrifuge tube. Add PBS Solution to a final volume of 200 µl. Vortex gently and incubate at room temperature for 1 min. Proceed to Step 2.

   1c. **Solidified Blood Clot**: Weigh 0.1 g of blood clot. Add liquid nitrogen and use a mortar/pestle to grind the sample into a fine powder. Add 200 µl of PBS Solution. Proceed to Step 2.

2. Add 20 µl of Proteinase K and mix by pipette. Add 200 µl of Buffer CL and vortex briefly. Incubate at 56°C for 10 min.
   •If solution is cloudy, continue incubation until clear.
   •If final reaction volume is >500 µl, increase Proteinase K usage and/or extend incubation.
   •If RNA-free genomic DNA is required, add 20 µl of 10 mg/ml RNase A (not included) vortex briefly and incubate at room temperature for 5 min. Proceed to Step 3.

3. Add 200 µl of 100% Ethanol and mix thoroughly by pipette.

4. Transfer the mixture into an EZ-10 Spin Column and incubate at room temperature for 1 min. Centrifuge for 2 min. Discard the flow-through.

5. Add 500 µl of CW1 Solution (with added ethanol) to the EZ-10 Spin Column and centrifuge for 1 min. Discard the flow-through.

6. Add 500 µl of CW2 Solution (with added ethanol) to the EZ-10 Spin Column and centrifuge for 1 min. Discard the flow-through.

7. Centrifuge for an additional 1 min to remove residual buffers.

8. Discard the Collection Tube and transfer the EZ-10 Spin Column to a new 1.5 ml centrifuge tube.

9. Add 30-50 µl CE Buffer to the center of the EZ-10 Spin Column. Incubate at room temperature for 2 min and centrifuge for 1 min. Store purified genomic DNA at -20°C.

General Notes

Storage of Blood Samples
This kit may be used on fresh or frozen whole blood samples that have been treated with EDTA, ACD or heparin. Blood should be collected in tubes containing the anticoagulant EDTA and stored at 2-8°C for short term (<10 days) or at -80°C for long term. To minimize risk of DNA degradation, it is recommended to store blood samples for no longer than 3 days.

Blood Collection and Treatment
For every 1 ml of whole blood sample, add 0.1 ml anticoagulant (0.5M EDTA pH 8.0, or ACD, 0.48% Citric Acid, 1.32% Sodium Citrate, 1.47% Glucose).

Troubleshooting
•Buffer CL may form a precipitate upon storage; dissolve by incubating at 40-50°C.
•Low yield: ensure Spin Column binding capacity of 10 µg is not exceeded.
•RNA contamination: RNase A activity is weak. Add 30% additional volume RNase A.
•Purified DNA samples do not settle into agarose gel: sample contains residual ethanol from wash steps. Ensure flow-through is discarded and repeat Step 7.

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com