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Column-Pure Blood Genomic DNA Mini Kit

Cat. No.	Product Name	Quantity
D203-Mini-1	Column-Pure Blood Genomic DNA Mini Kit	50 Preparations

Product Description
The **Column-Pure Blood Genomic DNA Mini Kit** is a quick and easy spin column method for isolation of genomic DNA from as little as 100 µl of whole blood. The purified DNA is suitable for molecular biology applications such as PCR, restriction digestion, and many more downstream applications.

Kit Contents

Component	Size
PBS Solution	8 ml
Buffer CL	12 ml
CW1 Solution (Concentrate)	13 ml
CW2 Solution (Concentrate)	9 ml
CE Buffer (pH 9.0)	15 ml
Buffer TBP (optional)	50 ml
Proteinase K	1.2 ml
Spin Columns	50 pieces

Storage of Kit Components
Store all Buffers/Solutions at room temperature; keep Proteinase K at -20 °C.

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

Anticoagulant components
- 0.5 M EDTA pH 8.0, or ACD
- 0.48% Citric Acid
- 1.32% Sodium Citrate
- 1.47% Glucose

For every 1 ml of whole blood sample, add 0.1 ml of anticoagulant.

Troubleshooting
- Low yield: Ensure column binding capacity of 10 µg is not exceeded.
- RNA contamination: RNase activity is weak/lost. Add 30% additional RNase A. Store at 4 °C.
- Sample floats when loading onto agarose gel: Sample contains ethanol from washing step. Discard liquid waste from collected after step 8. Spin again for additional 2 min. Incubate the column at 50 °C for ~5 mins to evaporate ethanol completely. Proceed to step 9.

Protocol

1. A. Before use, note that Buffer CL may form a precipitate upon storage; if necessary, dissolve the precipitate by warming to 56 °C.
   B. Before use, **add 17 ml of 100% ethanol** to the 13 ml CW1 Solution (Concentrate) and 21 ml of 100% ethanol to 9 ml of CW2 Solution (Concentrate).

2. Sample Preparation varies depending on the type of blood sample:
   A. **For non-nucleus Erythrocytes (e.g. Human Blood):** Harvest ~100 µl of blood into a 2.0 ml centrifuge tube. Add PBS solution to a final volume of 200 µl. Vortex gently and let stand for 1 min at room temperature. If >100 µl of blood used, add 2 volumes of Buffer TBP. Mix thoroughly and let stand for 1 min until to complete lysis. Spin at 4,000 x g (8,000 rpm) for 1 min. Discard the supernatant carefully. Wash the precipitate with 500 µl TE Buffer two times. Spin at 4,000 x g (8,000 rpm) for 1 min during each wash. The final precipitate should appear white. Typical yield is 1-3 µg from a 100 µl blood sample.
   B. **For nucleus-containing Erythrocytes (e.g. Chicken Blood):** Harvest ~10 µl of blood into a 2.0 ml centrifuge tube. Add PBS solution to a final volume of 200 µl. Vortex gently and let stand for 1 min at room temperature.
   C. **For solidified blood clot:** Weigh 0.1 g of blood. Grind to find powder under liquid nitrogen. Add 200 µl of PBS solution.

3. Add 20 µl of Proteinase K. Mix well. Add 200 µl of Buffer CL. Vortex Gently. Incubate at 56 °C for 10 min. If solution is cloudy, extend incubation until solution is clear to complete lysis. If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), vortex gently, and incubate for 5 min at room temperature before continuing to step 5. If final reaction volume is >500 µl, increase Proteinase K usage and/or extend incubation time.

4. Add 200 µl of 100% ethanol to the mixture and mix thoroughly. Small cloudy insoluble material may appear. This does not affect kit performance.

5. Transfer entire tube components into a column that is in a 2.0 ml Collection Tube. Let stand at room temperature for 1-2 min. Spin at 8,000 x g (10,000 rpm) for 2 min. Discard flow-through in the collection tube.

6. Add 500 µl of CW1 Solution. Spin at 8,000 x g (10,000 rpm) for 1 min.

7. Add 500 µl of CW2 Solution. Spin at 8,000 x g (10,000 rpm) for 1 min.

8. Discard the flow-through. Spin at 8,000 x g (10,000 rpm) for additional 1 min.

9. Place column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl CE Buffer into the center of the column's membrane. Incubate at room temperature for 2-3 min. To increase recovery yield, you may try incubating at 37 °C or 50 °C for 2 min.

10. Spin at 8,000 x g (10,000 rpm) for 1 min to elute DNA from column.

11. For long term storage, keep aliquots of purified DNA at -20 °C.

12. Measure DNA quantity by UV absorption at A_{260} (1.0 O.D. is equivalent to 50 µg). Assess DNA quality and yield via 0.7 % agarose gel.