√	If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 12.

11. Incubate at 55 ºC until the tissue is completely lysed (usually 1-3 hours). Vortex occasionally. Incubation in shaking water bath can reduce lysis time.

12. Cool to room temperature. Vortex for 20 seconds and centrifuge at 10,000 *x g* (12,000 *rpm*) for 5 minutes.

13. Pipette 300 µl of supernatant to a new Eppendorf tube, add 300 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution to a EZ-10 Spin Column.

14. Centrifuge at 2,000 *x g* (4,000 *rpm*) for 2 minutes and discard the flow-through.

15. Add 500 µl of Wash Solution, and spin at 6,000 *x g* (8,000 *rpm*) for 1 minute.

16. Repeat Step 15.

17. Discard the flow-through and spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove residual amount of Wash Solution.

18. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

19. Spin at 8,000 *x g* (10,000 *rpm*) for 1 minute to elute DNA from the column.

20. For long term storage, keep aliquots of purified genomic DNA at -20 ºC.

21. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel.

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EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood (New!)

Kit Contents

Component	SK8253, 50 Preps	SK8254, 100 Preps
PBS Solution	8 ml	16 ml
Buffer CL[(a)]	12 ml	24 ml
CW1 Solution(Concentrate) [(b)]	13 ml	26 ml
CW2 Solution(Concentrate) [(b)]	9 ml	18 ml
CE Buffer (pH 9.0)	15 ml	30 ml
Buffer TBP (optional)	50 ml	100 ml
Proteinase K[(c)]	1.2 ml	2.4 ml
EZ-10 Column (with 2.0-ml Collection Tube)	50	100
Protocol	1	1

Note:

a. Buffer CL Solution may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution to 56ºC.
b. Before use, add 17 ml of 100% ethanol to 13 ml CW1 Solution (Concentrate) and 21 ml of 100% ethanol to 9 ml of CW2 Solution (Concentrate) for SK8253; add 34 ml of 100% ethanol to 26 ml CW1 Solution (Concentrate) and 42 ml of 100% ethanol to 18 ml of CW2 Solution (Concentrate) for SK8254.
c. Proteinase K is supplied in liquid and is stable at Room temperature. For long term storage, please keep at -20ºC.
d. CE Buffer is 10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0. Although TE buffer pH 8.0 or water can be used, yield is generally 20% lower.

Storage of Blood

Whole blood samples treated with EDTA, ACD or heparin can be used, and may be either fresh or frozen. For short term storage (up to 10 days), collect blood in tubes containing EDTA as an anticoagulant, and store tubes at 2-8ºC. It is

recommended to store blood samples less than 3 days as DNA degradation may occur. For long term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA if high molecular weight DNA is required) and store at -80ºC.

Blood Collection and Treatment

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

Procedure for Extraction Genomic DNA from Blood

1. Sample Preparation

A. Blood Samples (non-nucleated Erythrocytes, for example Human Blood): Collect ~100ul of blood into 2.0 ml centrifuge tube. Add PBS solution to the tube to a final volume of 200ul. Vortex gently and let the tube stand for 1 min at room temperature.

√	If >100ul of blood is used, add 2 volumes of Buffer TBP. Mix thoroughly and let the tube stand for 1 min until red cells lyse completely. Spin at 4,000 Xg (8,000 rpm) for 1 min. Discard the supernatant carefully. Wash the precipitate with 500ul TE Buffer 2 times. Spin at 4,000 Xg (8,000 rpm) for 1 min during each wash. The final precipitate should appear white. Proceed with step 2.
√	Typical Yield is 1-3ug from 100ul blood sample.

B. Blood Samples (Nucleus-containing Erythrocytes, for example chicken Blood): Collect ~10ul of blood into a 2.0 ml centrifuge tube. Add PBS solution to the tube to a final volume of 200ul. Vortex gently and let the tube stand for 1 min at room temperature. Proceed to step 2.

C. Solidified Blood Clot: Weigh 0.1g of blood. Grind to find powder under liquid nitrogen. Add 200ul of PBS solution and proceed to step 2.

2. Add 20ul of proteinase K. Mix well. Add 200 ul of Buffer CL. Vortex Gently. Incubate at 56ºC for 10min.

√	The solution should appear clear after complete lysis. If solution still appears cloudy, please extend incubation time until lysis is complete and solution is clear.
√	If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 3.
√	If final reaction volume is more than 500ul, please increase proteinase K usage and/or extend incubation time.

3. Add 200ul of 100% ethanol to the mixture and mix thoroughly.

√	Small cloudy insoluble material may appear after addition of ethanol, but this does not affect the performance of the kit. Proceed with step 4.

4. Transfer the mixture from step 3 (including any precipitates) into an EZ-10 column that is in a 2.0 ml Collection Tube. Let it stand at Room Temperature for 1-2min. Spin at 8,000 *x g* (10,000 *rpm*) for 2 minutes. Discard the flow-through in the collection tube.

5. Add 500 µl of CW1 Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 1 minute.

√	Please ensure ethanol has been added to the CW1 concentrate prior to usage

6. Add 500 µl of CW2 Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 1 minute.

√	Please ensure ethanol has been added to the CW2 concentrate prior to usage

7. Discard the flow-through. Spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove any residual amount of CW2 Solution.

8. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl CE Buffer into the center part of membrane in the column. Incubate at RT for 2 to 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

9. Spin at 8,000 *x g* (10,000 *rpm*) for 1 minute to elute DNA from the column.

10. For long term storage, keep aliquots of purified genomic DNA at -20 ºC. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel.

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Troubleshooting Guide: EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood

Low yield

There are a number of variables that can cause low yield.
a. Each step has to be strictly followed.
b. Make sure column binding capacity of 10 µg is not exceeded.

RNA contamination

RNase activity is weakened or lost. Add 30% additional RNase A, and store solution at 4ºC.

Sample floats upon loading in agarose gel

The sample contains ethanol from washing step. Discard the liquid waste from the collection tube after washing step, and spin again for additional two minutes. Before elution step, incubate the column at 50ºC for ~5 min and allow ethanol to evaporate completely.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!