PURO
Blood DNA

For isolation of genomic DNA

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PURO-Blood DNA

(Spin Column)

Cat. no. SC05

Kit Contents

Contents	DP318-02 50 preps	DP318-03 200 preps
Buffer CL (Red Cell Lysis)	60 ml	250 ml
Buffer GS	15 ml	50 ml
Buffer GB	15 ml	50 ml
Buffer GD	13 ml	52 ml
Buffer PW	15 ml	50 ml
Buffer TB	15 ml	60 ml
Proteinase K	1 ml	4 × 1 ml
Spin Columns CB3	50	200
Collection Tubes 2 ml	50	200
Collection Tubes 1.5 ml	50	200
Handbook	1	1

Compatible Reagents

RNase A (100 mg/ml)

Storage

PURO Blood DNA Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C. For longer periods, storing the PURO Blood DNA Kit at 2–8°C is recommended.

Introduction

PURO Blood DNA Kit use silica membrane technology and special buffer system for Blood gDNA extraction effectively. The spin column made of new type silica-gel membrane in blood DNA kit can be easily bounded by DNA specifically. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure DNA to be eluted in either water or a buffer provided with the kit.

The genomic DNA isolated with these products is of high quality and serves as an excellent template for agarose gel analysis, restriction enzyme digestion, PCR analysis and blotting procedures.

Yield :

Sample	Volume	DNA yield
Whole Blood from Mammal	100 µl-1 ml	3-30 µg
Whole Blood from birds or amphibian	5—20 µl	5-40 µg

Important Notes

1. Samples should not be frozen and thawed more than once. Equilibrate samples to room temperature (15–25°C).
2. If precipitates have formed in Buffer GS or Buffer GB, dissolve them by incubating at 56°C.
3. Heat a water bath or heating block to 56°C.
4. Ensure Buffer GD and Buffer PW have been prepared with appropriate amount of ethanol (96–100%).

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly. This kit is designed for processing blood sample of 0.1-1 ml.

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1. Preparation of samples

a. Pipet 200 µl sample to the microcentrifuge tube. If the volume is less than 200 µl, adjust volume to 200 µl with buffer GS.

b. If the sample volume is more than 200 µl (e.g. 300 µl - 1 ml), please refer the following step: add 1-2.5 times volume Red Cell Lysis Buffer (Buffer CL) to the sample, mix by inverting the tube, and close the cap. Incubate the tube in room temperature (15–25°C) for 5 min, and centrifuge at 10,000 rpm (~11,500 × g) for 1 min, then discard the flow-through. (If the lysis is not complete, repeat the lysis steps once.) Pipet 200 µl buffer GS and mix by pulse-vortex.

c. If the sample is blood from poultry, birds, amphibians, of which red blood cells have nucleolus, the amount should be reduced to 5-20 µl and adjust the volume to 200 µl with buffer GS.

Note: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml, should be prepared by user). Mix by vortex for 15 s, and incubate for 5 min at room temperature (15–25°C).

2. Add 20 µl Proteinase K, mix thoroughly by vortex.
3. Add 200 µl Buffer GB to the sample, mix thoroughly by vortex, and incubate at 56°C for 10 min to yield a homogeneous solution. (If the mixture does not become clean, extend the incubation time until a homogeneous solution is obtained.)

Note: White precipitates may form when Buffer GB is added. They will not interfere with the procedure and will dissolve during the heat incubation at 56°C. If precipitates do not dissolve during heat incubation, it indicates that the cell is not completely lysed and may result in low yield of DNA and impurity in DNA.

4. Add 200 µl ethanol (96-100%) to the sample, and mix

thoroughly by vortex for 15 s. A white precipitate may form on addition of ethanol.

5. Pipet the mixture from step 4 into the Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the spin column into the collection tube.
6. Add 500 µl Buffer GD (ensure ethanol has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
7. Add 700 µl Buffer PW (ensure ethanol has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
8. Add 500 µl Buffer PW to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
9. Set the Spin Column CB3 back to the Collection Tube and centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely. Discard the flow-through, and allow the column to air dry with the cap open for several minutes to dry the membrane.

   Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with down-stream reactions.
10. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 min, and

then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

Note: The volume of elution buffer should not be less than 50µl, or it may affect the recovery efficiency. What's more, the pH value of elution buffer have influence in eluting, we suggest use buffer TE or distilled water (pH 7.0-8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer TE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.