EasyPrep Blood DNA Purification Kit

For isolation of gDNA from 0.1–1 mL whole blood

Contents

Introduction

EasyPrep Blood DNA Purification Kit uses a spin column that can specifically bind to DNA and provides a special buffer system for effective blood gDNA extraction. The spin column is composed of a novel silica membrane that binds to DNA efficiently and specifically. It can remove contaminant proteins and other organic compounds in cells. gDNA isolated using this product is highly pure, stable, and integrated. gDNA isolated using this product can be used as a template for downstream techniques such as restriction enzyme digestion, PCR analysis, library construction, Southern blot procedures, chip hybridization, and high-throughput sequencing.

Features

1. Wide application: The kit can be used to extract gDNA directly from anticoagulant blood (e.g., EDTA and heparin), buffy coat, and blood clot.
2. High quality: With the unique lysis buffer system, the purified DNA with high concentration, purity, and integrality can satisfy the requirements of chip hybridization and high-throughput sequencing.
3. Rapid and nontoxic: The kit uses silica membrane adsorption technology and does not need phenol and chloroform. The whole extraction process can be completed within an hour.

Yield

Sample	Volume	DNA yield
Whole blood from a mammal	100 µL – 1 mL	3–30 µg
	500–1000	10–30 µg
Whole blood from a bird or amphibian	5-20	5–40 µg
	200-500	1–8 µg
Blood clots	500-1000	8–15 µg

Note:

1. Samples should not have been frozen and thawed more than once. Equilibrate samples to room temperature (15–25 °C).
2. Buffer PCB should be prepared with 60 mL of ethanol (96%–100%).

Kit Contents

Contents	DPT-BC48 (50 preps)
Buffer CCL	60 mL
Buffer GCS	15 mL
Buffer GCB	15 mL
Buffer BCD	20 mL
Buffer CDB	30 mL
Buffer PCB	15 mL
Buffer EB	15 mL
Proteinase K	1 mL
Spin Columns	50
Collection Tubes (2 mL)	50
Collection Tubes (1.5 mL)	50

Storage

EasyPrep Blood DNA Purification Kit can be stored dry at room temperature (15–25 °C) for up to 12 months without any reduction in performance and quality.

Note: Reagent not supplied:

1. RNase A (TE- RA100)
2. Liquid column CCX1 (RKT-BA65)

Protocol

Note: Buffer PCB should be added to 60 mL of ethanol (96%–100%) as indicated on the bottle followed by thorough shaking. This kit is designed for processing blood samples of 0.1–1 mL.

1. Preparation of mammalian blood samples (this kit is designed for a 0.1–1 mL blood sample):

a. For 200 µL of mammalian blood, proceed directly to the next step.

b. For <200 µL of mammalian blood, adjust the volume to 200 µL with Buffer GCS.

Note: Protocols a and b could be applied for the extraction of a 100–200-µL blood sample; however, blood samples with high protein, saccharides, and lipid content or maintained under poor storage condition may provide a low OD260/OD230 ratio. Adding 1–2.5 times the sample volume of Buffer CCL could increase the ratio of OD260/OD230.

c. For >200 µL of human blood, add 1–2.5 times the volume of Buffer CCL to the sample and mix by inverting the tube. Centrifuge at 10,000 rpm (~11,500 ×g) for 1 min and then discard the supernatant (if the lysis is not completed, add 1–2.5 times the volume of Buffer CCL and repeat the lysis steps once). Add 200 µL Buffer GCS and mix thoroughly on a vortex mixer.

d. For avian or amphibian blood samples (which contain red blood cells with nucleoli), reduce the sample amount to 5–20 µL and adjust the volume to 200 µL by adding Buffer GCS.

e. For blood clots, use liquid column CCX1 (TOOLS Cat no. RKT-BA65, not provided) to liquefy the sample. The steps are as follows:

e1. Transfer blood clots into liquid column CCX1, centrifuge at 12,000 rpm (~11,500 × g) for 1 min, and then collect the filtrate (for large volumes of clots, divide the sample and centrifuge several times, and then collect the filtrate).

e2. Transfer 100 µL to 1 mL of filtrate and add 1–2.5 times the volume of Buffer CCL, mix by inverting, centrifuge at 10,000 rpm (~11,500 ×g) for 1 min, and then, discard the supernatant (if the lysis is not complete, add 1–2.5 times the volume of Buffer CCL and repeat the lysis steps once). Add 200 µL Buffer GCS and mix thoroughly on a vortex mixer.

Note: If RNA-free gDNA is required, add 4 µL RNase A (100 mg/mL, not provided). Mix on a vortex mixer for 15 sec, and incubate for 5 min at room temperature (15–25 °C).

2. Add 200 µL of Buffer GCB and 20 µL of Proteinase K to the sample, mix thoroughly on a vortex mixer , and incubate at 56 °C for 10 min to form a homogeneous solution (if the mixture does not become clear, extend the incubation time until a homogeneous solution is obtained).

Note:

1. White precipitates may form when Buffer GCB is added. These would disappear when incubated at 37 °C and would not influence downstream experiments. If the solution does not become clear, the cells are not completely lysed; the consequent results may have a low yield and purity. If the sample volume is >200 µL and the Buffer CCL lysis step is not taken or if the storage condition is poor, the color may become dark brown after being placed in a hot water bath; no block

precipitates should remain in the solution.

2. If the blood sample has been treated by Buffer CCL, add Buffer GCB and mix by inverting, and then incubate for 5 min at room temperature; subsequently, high-quality gDNA can be isolated.

3. Add 350 µL of Buffer BCD to the sample after incubating for 2–5 min at room temperature (15–25 °C); mix by inverting and flocky precipitates may form.
4. Pipet the mixture including flocky precipitates from Step 3 into the spin column (in the collection tube) and centrifuge at 12,000 rpm (~13,400 ×g) for 30 sec. Discard flow-through and place the spin column into the collection tube.
5. Add 500 µL of Buffer CDB to the spin column and centrifuge at 12,000 rpm (~13,400 ×g) for 30 sec, and then discard the flow-through and place the spin column into the collection tube.
6. Add 600 µL of Buffer PCB (ensure ethanol (96%–100%) has been added) to the spin column and centrifuge at 12,000 rpm (~13,400 ×g) for 30 sec. Discard the flow-through and place the spin column into the collection tube.
7. Repeat Step 6.

Note: If the blood sample has been treated by Buffer CCL, skip Step 8.

8. 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 dry for several minutes to dry the membrane.

Note: Step 8 is for removing the residual buffer on the spin column. The residual ethanol of the buffer will influence the downstream enzyme reaction (e.g., enzyme digestion and PCR).

9. Place the spin column in a clean 1.5-mL microcentrifuge tube, and pipet 50–200 µL Buffer EB to the center of the membrane. Incubate at room temperature (15–25 °C) for 2 min and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

Note: The volume of the elution buffer should not be <50 µL, or it may affect the recovery efficiency. Add the solution to the spin column after centrifuging, incubate at room temperature (15–25 °C) for 2 min and centrifuge for 2 min at 12,000 rpm (~13,400 × g). The pH of the elution buffer has a substantial impact on eluting; we suggest using ddH2O (pH 7.0–8.5) to elute gDNA. The isolated gDNA should be stored at −20 °C to prevent degradation.

This product is for research only, not for diagnostic and clinical use.