EasyPrep Genomic DNA Extraction Kit

For isolation of genomic DNA from blood, cells and animal tissues

Contents

Introduction

EasyPrep Genomic DNA Kit is used to extract many types of gDNA. It is based on silica membrane technology and a special buffer system. The spin column is composed of a new type of silica membrane that can bind DNA optimally under given salt and pH conditions. Simple centrifugation completely removes contaminants and enzyme inhibitors, such as proteins and divalent cations. The purified DNA is eluted in water or a low-salt buffer and is ready for use in downstream applications.

DNA purified by the Kit is highly suited for restriction analysis, PCR analysis, southern blotting, and cDNA-library applications.

Kit Contents

Contents	DPT-BC04 50 preps	DPT-BC04-2 200 preps
Buffer GBA	15 mL	50 mL
Buffer GBB	15 mL	50 mL
Buffer GBD	13 mL	52 mL
Buffer PBW	15 mL	50 mL
Buffer TE	15 mL	60 mL
Proteinase K	1 mL	4×1 mL
Spin Columns CB3	50	200
Collection Tubes（2 mL）	50	200
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Storage

The EasyPrep Genomic DNA Kit can be stored dry at room temperature (15°C–25°C) for up to 12 months with no detriment to performance and quality.

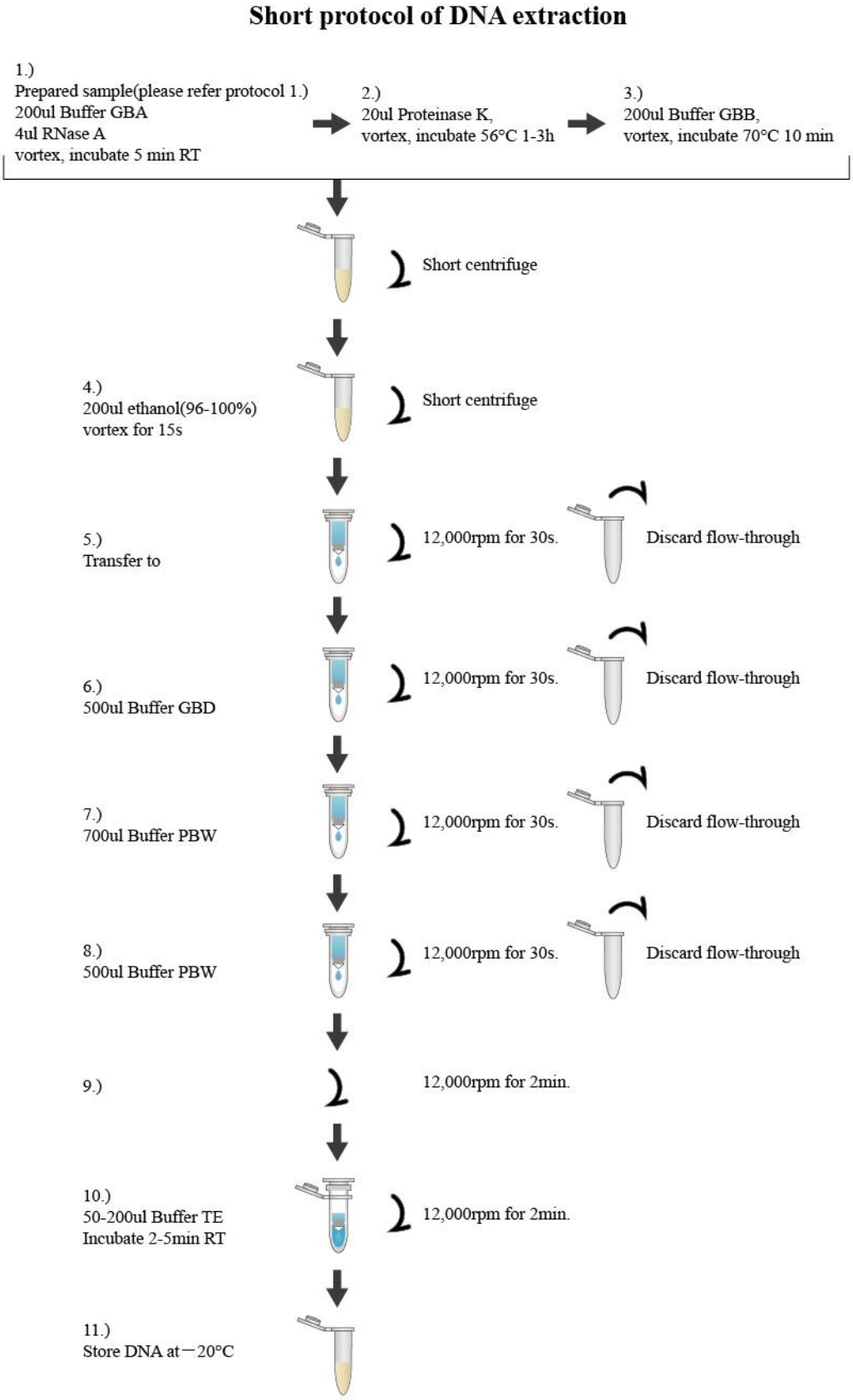
Genomic DNA Yield from the EasyPrep Genomic DNA Kit

Source	DNA Yeild
Whole blood from mammalian (100 μL-400 μL)	3-10 μg
Whole blood from bird or amphibian (5-20 μL)	5-40 μg
Cultured cells (10^6-10^7cells)	5-30 μg
Tissue (30 mg)	10-30 μg

Important Notes

1. The repeated freezing and thawing of stored samples should be avoided because this reduces DNA size.
2. If a precipitate has formed in Buffer GBA or Buffer GBB, warm the buffer to 56℃ until the precipitate has fully dissolved.
3. All centrifugation steps should be executed in a conventional table-top microcentrifuge at room temperature (15°C–25°C).

Work Flow


Short protocol of DNA extraction
1.)
Prepared sample(please refer protocol 1.)
200ul Buffer GBA
4ul RNase A
vortex, incubate 5 min RT
2.)
20ul Proteinase K,
vortex, incubate 56°C 1-3h
3.)
200ul Buffer GBB,
vortex, incubate 70°C 10 min
Short centrifuge
4.)
200ul ethanol(96-100%)
vortex for 15s
Short centrifuge
5.)
Transfer to
12,000rpm for 30s.
Discard flow-through
6.)
500ul Buffer GBD
12,000rpm for 30s.
Discard flow-through
7.)
700ul Buffer PBW
12,000rpm for 30s.
Discard flow-through
8.)
500ul Buffer PBW
12,000rpm for 30s.
Discard flow-through
9.)
12,000rpm for 2min.
10.)
50-200ul Buffer TE
Incubate 2-5min RT
12,000rpm for 2min.
11.)
Store DNA at－20°C

Protocol

Note: Ensure that Buffer GBD and Buffer PBW have been prepared with the appropriate volume of ethanol (96%–100%), as indicated on the bottle, and shake thoroughly.

1. Prepare the samples. For blood with nonnucleated erythrocytes, follow step a; for blood with nucleated erythrocytes, follow step b; for cultured cells, follow step c; for tissue, follow step d.

a. For non-nucleated applications: pipette 200 µL of the sample to the microcentrifuge tube. If the sample volume is less than 200 µL, adjust the volume to 200 µL using Buffer GBA. If the sample volume is more than 200 µL, (e.g., from 300 µL to 1 mL), please refer the following steps. Add three times the volume of Red Cell Lysis Buffer to the sample, invert the tube and close the cap, allow the tube to stand at room temperature (15°C–25°C) for 5 min, centrifuge at 12,000 rpm (≈13,400 × g) for 1 min, discard the flow-through, pipette 200 µL of Buffer GBA into the tube, and finally mix by pulse vortexing.

b. For nucleated applications: add 5–20 µL of anticoagulated blood and adjust the volume to 200 µL using Buffer GBA.

c. For cultured cells: centrifuge the cells (10^6 to 10^7cells) for 1 min at 12,000 (≈13,400 × g), discard the flow-through, and finally resuspend the cell pellet in 200 µL of Buffer GBA.

d. For tissue: Cut no more than 25 mg of tissue (spleen tissue <10 mg) into small pieces, place the tissue in a 1.5mL microcentrifuge, centrifuge at 12,000 rpm (≈13,400 × g) for 1 min, discard the supernatant, and finally resuspend the cell pellet in 200 µL of Buffer GBA.

Optional: For the RNase treatment of the sample, add 4 µL of RNase A (100mg/mL), mix by vortexing, and finally incubate for 5 min at room temperature (15°C–25°C).

2. Add 20 µL of proteinase K and mix thoroughly by vortexing. If the sample is tissue, incubate at 56°C until the tissue is completely lysed.

Note: Lysis time varies depending on the tissue type processed. Lysis is usually complete in 1–3 h. For convenience, samples can be lysed overnight with no adverse effects.

3. Add 200 µL of Buffer GBB to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5-mL microcentrifuge tube to remove drops from the inside of the lid.
4. Add 200 µL of ethanol (96%–100%) to the sample and mix thoroughly by vortexing for 15 s. A white precipitate may form upon the addition of ethanol. Briefly centrifuge the 1.5-mL microcentrifuge tube to remove drops from the inside of the lid.
5. Pipette the mixture from step 4 into the EasyPrep Spin Column CB3 (in a 2-mL collection tube) 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.

6. Add 500 µL of Buffer GBD to EasyPrep Spin Column CB3, centrifuge at 12,000 rpm (≈13,400 × g) for 30 s, and finally discard the flow-through and place the spin column into the collection tube.
7. Add 700 µL of Buffer PBW to EasyPrep Spin Column CB3, centrifuge at 12,000 rpm (≈13,400 × g) for 30s, discard the flow-through, and finally place the spin column into the collection tube.
8. Add 500 µL of Buffer PBW to EasyPrep Spin Column CB3, centrifuge at 12,000 rpm (≈13,400 × g) for 30 s, discard the flow-through, and finally place the spin column into the collection tube.
9. Centrifuge at 12,000 rpm (≈13,400 × g) for 2 min to dry the membrane completely.

Note: The resident ethanol of buffer PBW may partially affect downstream applications.

10. Place the EasyPrep Spin Column CB3 in a new and clean 1.5-mL microcentrifuge tube and pipette 50–200 µL of distilled water or Buffer TE directly to the center of the membrane. Incubate at room temperature (15°C–25°C) for 2–5 min and then centrifuge for 2 min at 12,000 rpm (≈13,400 × g).

Note: If the volume of the eluted buffer is less than 50 µL, recovery efficiency may be affected. The pH value of the eluted buffer also partially affects elution. Thus, we recommend using Buffer TE or distilled water (pH: 7.0–8.5) for gDNA elution.

For long-term DNA storage, elution in Buffer TE and storage at −20°C is recommended because if stored in water, DNA is susceptible to acid hydrolysis.