EasyPure® Genomic DNA Kit

Cat. No. EE101

Version No. Version 2.0

Storage: at room temperature (15-25°C) in a dry place for one year.

Description

EasyPure® Genomic DNA Kit provides a simple and convenient way to efficiently isolate high-quality genomic DNA from a variety of materials (mammalian cells, tissues, mouse tails, *E. coli* and yeast). Cells and tissues are enzymatically lysed. DNA is specifically bound to silica-based column. The isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot, *etc*.

- Fast extraction, high yield (up to 15 μg).
- High integrity of cDNA ensured by mild lysis conditions without physical disruption, thus reducing damage to gDNA during cell lysis process
- High purity enabled by spin column which can efficiently and specifically bind to DNA and removs protein, salts, lipids or other contaminants.

Kit Contents

Component	EE101-01 (50 rxns) EE101-11 (50 rxns)	EE101-02 (200 rxns) EE101-12 (200 rxns)
Lysis Buffer 2 (LB2)	6 ml	24 ml
Binding Buffer 2 (BB2)	28 ml	110 ml
Clean Buffer 2 (CB2)	15 ml	60 ml
Wash Buffer 2 (WB2)	12 ml	2×22 ml
Elution Buffer (EB)	25 ml	80 ml
RNase A (20 mg/ml)	1 ml (EE101-01) 0 (EE101-11)	4×1 ml (EE101-02) 0 (EE101-12)
Proteinase K (20 mg/ml)	1 ml	4×1 ml
Genomic Spin Column with Collection Tubes	50 each	200 each

Sample requirement

Material	Amount
Mammalian Cell	1-5×10^6 cell
Mammalian Tissues	≤25 mg
Mouse Tail	0.5 cm sections
E. coli Cells	≤2×10^9 cell
Yeast Cells	≤5×10^7 cell

Procedures

Before starting, adding different volumes of 100% ethanol to CB2 and WB2.

Component	EE101-01/11 (50 rxns)	EE101-01/11 (200 rxns)
Clean Buffer 2 (CB2)	15 ml	60 ml
Wash Buffer 2 (WB2)	48 ml	2×88 ml

All centrifugation steps are carried out at room temperature.

1. Processing materials

• Mammalian Cells

a) Adherent cells: Remove the culture media from culture plate and harvest cells by trypsin or other methods. Collect cells by centrifuging at 250×g for 5 minutes. Remove the supernatant.

b) Suspension cells: Harvest cells by centrifuging at 250×g for 5 minutes. Remove the supernatant.

c) Add 100 μl of LB2 to the cell pellet. Mix thoroughly by vortexing or pipetting.

Optional: If RNA-free genomic DNA is required, add 20 μl of RNase A to the lysate, and incubate at room temperature for 2 minutes.

d) Add 20 µl of Proteinase K to the lysate. Mix well by vortexing, and then incubate at room temperature for 2 minutes.

• Mammalian Tissues

Prepare 55°C water bath or metal bath before starting.

a) Transfer ≤25 mg (spleen≤10 mg) chopped tissue to a sterile 1.5 ml microcentrifuge tube.

b) Add 100 µl of LB2 and 20 µl of Proteinase K to the tube. Make sure that the tissue is completely immersed in the tube.

c) Incubate at 55°C until the sample is completely lysed (3 hours are needed for most tissues; 6-8 hours or longer are needed for mouse tail. Overnight incubation is needed if necessary. Invert the lysate 2~3 times every hour).

Optional: If RNA-free genomic DNA is needed, add 20 µl of RNase A to the lysate, incubate at room temperature for 2 minutes.

d) Centrifuge at 12,000×g for 5 minutes, and transfer the supernatant to a sterile 1.5 ml microcentrifuge tube.

• *E. coli* Cells

Prepare 55°C water bath or metal bath before starting.

a) Transfer 1~5 ml of bacteria culture to a 1.5 ml tube and centrifuge the tube at 12,000×g for 1 minute. Discard the supernatant as thoroughly as possible.

b) Add 100 µl of LB2 and 20 µl of Proteinase K into the tube. Resuspend the cell pellet thoroughly by vortexing.

c) Incubate at 55°C for 15 minutes.

Optional: If RNA-free genomic DNA is needed, add 20 µl of RNase A to the sample, and incubate at room temperature for 2 minutes.

• Yeast Cells

□ Prepare 37°C and 55°C water bath or metal bath before starting

□ Prepare fresh sorbitol buffer (1 M sorbitol, 10 mM EDTA, 14 mM β-mercaptoethanol).

□ Prepare lyticase.

a) Harvest yeast cells (≤5×10^7 cells) by centrifuging at 12,000×g for 1 minute. Discard the supernatant as thoroughly as possible.

b) Add 500 µl of sorbitol buffer and 15 units of lyticase to the pellet. Mix thoroughly and incubate at 37°C for 1 hour.

c) Centrifuge at 5,000×g for 10 minutes. Discard the supernatant.

d) Resuspend pellets by adding 100 µl of LB2 and 20 µl of Proteinase K. Mix thoroughly by vortexing.

e) Incubate at 55°C for 45 minutes.

f) Centrifuge at 12,000×g for 5 minutes and transfer the supernatant to a sterile 1.5 ml microcentrifuge tube.

Optional: If RNA-free total DNA is needed, add 20 µl of RNase A to the lysate, and incubate at room temperature for 2 minutes.

2. Add 500 µl of BB2, and immediately mix by vortexing for 5 seconds. Incubate at room temperature for 10 minutes.

3. Transfer all the lysate to a spin column. Centrifuge at 12,000×g for 30 seconds. Discard the flow through.

4. Add 500 µl of CB2 (check to ensure you have added ethanol prior to use) and centrifuge at 12,000×g for 30 seconds. Discard the flow through.

5. Add 500 µl of WB2 (check to ensure you have added ethanol prior to use) and centrifuge at 12,000×g for 30 seconds. Discard the flow through.

6. Repeat step 5 once.

7. Centrifuge at 12,000×g for 2 minutes to remove residual WB2 thoroughly.

8. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 µl of Elution Buffer (preheated to 60-70°C) or sterile, deionized water (pH >7.0, preheated to 60-70°C) to the center of column matrix. Incubate at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute to elute DNA.

9. To improve the yield, repeat step 8 once. Store the isolated DNA at -20°C.

Notes

• It is important not to overload the column, otherwise the extraction result may be affected.
• Cut the tissue into pieces as small as possible. The lysate looks sticky, not gelatinous after complete lysis.
• To ensure high quality of the isolated DNA, use fresh materials and avoid repeated freezing and thawing. The quality of DNA depends on the type of material and storage time.
• Use sterile tubes and pipette tips to avoid DNase contamination.
• Use the same tube for the first round of elution or a new tube for the second round of elution.

For research use only, not for clinical diagnosis.

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Service telephone +86-10-57815020
Service email complaints@transgen.com