EasyPure® Genomic DNA Kit

Cat. No. EE101

Storage: RNase A and Proteinase K solutions at -20°C for two years; others at room temperature (15-25°C) for one year.

Description

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

- DNA yield up to 15 μg.
- Complete removal of contaminants and inhibitors.
- Column based purification, no organic extraction or ethanol precipitation.

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

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
Wash Buffer 2 (WB2)	12 ml	2×22 ml
Binding Buffer 2 (BB2)	28 ml	110 ml
Clean Buffer 2 (CB2)	55 ml	2×110 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

Before starting, adding appropriate volume of 96-100% ethanol to WB2.

WB2	12 ml	2×22 ml
Ethanol	48 ml	2×88 ml

All centrifugation steps are carried out at room temperature.

Procedures

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

• Mammalian Tissues

(Prepare 55°C water bath or heater 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; mix 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, transfer the supernatant to a sterile 1.5 ml microcentrifuge tube.

• Bacteria

(Prepare 55°C water bath or heater before starting).
a) Transfer 1~5 ml of cell culture (≤2×10^9) to a 1.5 ml tube and centrifuge the tube at 12,000×g for 1 minute. Discard the supernatant.
b) Add 100 µl of LB2 and 20 µl of Proteinase K into the tube. Resuspend the cell pellet by vortexing or pipetting.
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, incubate at room temperature for 2 minutes.

• Yeast Cells

(Prepare 37°C, 55°C water bath or heater before starting)
Prepare fresh D-glucitol buffer (1 M sorbitol, 10 mM EDTA, 14 mM β-mercaptoethanol). Prepare lyticase.
a) Harvest yeast cells (≤5×10^7) by centrifuging at 12,000×g for 1 minute. Discard the supernatant.
b) Add 500 µl of D-glucitol buffer, 15 units 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 in 100 µl of LB2 and 20 µl of Proteinase K, mix thoroughly by votexing.
e) Incubate at 55°C for 45 minutes.
Optional: If RNA-free total DNA is needed, add 20 µl of RNase A to the lysate, incubate at room temperature for 2 minutes.
f) Centrifuge at 12,000×g for 5 minutes and transfer the supernatant to a sterile 1.5 ml microcentrifuge tube.

2. Add 500 µl of BB2, mix by vortexing for 5 seconds, incubate at room temperature for 10 minutes.
3. Centrifuge the tube briefly and 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, Centrifuge at 12,000×g for 30 seconds. Discard the flow through.
5. Repeat step 4 once.
6. Add 500 µl of WB2 (check to ensure you have added ethanol) and centrifuge at 12,000×g for 30 seconds. Discard the flow through.
7. Repeat step 6 once.
8. Centrifuge the empty column at maximum speed (≥12,000×g) for 2 minutes to remove residual WB2.
9. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 µl of Elution Buffer (preheated to 65°C) or sterile, distilled water (pH >7.0, preheated to 65°C) to the column matrix. Incubate at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute to elute the isolated genomic DNA. Optional: To get more DNA by repeating step 9 once.
10. Store the isolated DNA at -20°C.

Notes

• It is important not to overload the column, as this can lead to significantly lower yields than expected.
• Cut the tissue as small pieces as possible. The complete lysate looks sticky, not gelatinous.
• Use fresh material and avoid repeated freezing and thawing .
• Use sterile tubes and pipette tips to avoid DNase contamination.

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