

EasyPure® Micro Genomic DNA Kit

Cat. No. EE181

Storage: Proteinase K solutions and Carrier RNA at -20°C for one year; others at room temperature (15-25°C) for one year

Description

EasyPure® Micro Genomic DNA Kit uses enzyme digestion method to lyse samples. The unique lysis buffer in this kit can efficiently lyse small volume of cells from a variety of materials including blood, dried blood spots, serum/plasma, mouthwash, hair follicles, tissues, microdissected tissues. DNA from the lysate will bind to silica-based column and elute with elution buffer. The isolated DNA is suitable for PCR, restriction enzyme digestion, and other downstream applications.

Kit Contents

Component	EE181-01 (50 rxns)
Lysis Buffer 14 (LB14)	6 ml
Binding Buffer 14 (BB14)	28 ml
Clean Buffer 14 (CB14)	28 ml
Wash Buffer 14 (WB14)	12 ml
Elution Buffer (EB)	5 ml
Carrier RNA (1 µg/µl)	55 µl
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Column with Collection Tubes	50 each

Sample Requirement

Material	Amount
Cultured cells	1×10 ⁴ -10 ⁶ cells
Tissues	≤10 mg
Microdissected tissues	≤10 mg
Formalin fixed tissues	≤10 mg
<i>E. coli</i>	≤1×10 ⁹ cells
Anti-coagulant blood	1-50 µl
Serum/plasma	50-250 µl
Mouthwash	2-20 ml
Dried blood spots	5 mm ² -100 mm ²
Hair follicles	1-20 pieces

Procedures

Before starting, add 48 ml of 96-100% ethanol to the bottle of WB14.

Equilibrate a water bath to 55°C. All centrifugation steps are carried out at room temperature.

1. Materials

• Cultured Cells

(a) For adherent cells, remove media from culture dish, harvest cells by Trypsin digestion or other cell detaching methods.

Centrifuge at 250×g for 5 minutes, discard the supernatant.

- (b) For suspension cells, harvest cells, and centrifuge at 250×g for 5 minutes, discard the supernatant.
- (c) Add 100 µl of LB14 to the cell pellets, mix thoroughly to resuspend the cells.
If RNA removal is needed, add 20 µl of RNase A to the sample, incubate at room temperature for 2 minutes.
- (d) Add 20 µl of Proteinase K to the sample, mix by vortexing and incubate at room temperature for 2 minutes.
- Tissues, Microdissected Tissues and Hair Follicles
 - (a) Add ≤10 mg of minced tissue or 1-20 pieces of 0.5 cm length of hair follicles from the bottom of hair into a 1.5 ml microcentrifuge tube.
 - (b) Add 100 µl of LB14 and 20 µl of Proteinase K to 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; one hour is needed for hair follicles; mix the lysate 2-3 times every hour.)
If RNA removal is needed, add 20 µl of RNase A to the sample, incubate at room temperature for 2 minutes.
 - (d) Centrifuge at 12,000×g for 5 minutes, gently transfer the supernatant to a sterile microcentrifuge tube.
- Bacteria
 - (a) Add 50 µl-1 ml of bacterial culture ($OD_{600}=1$) into a microcentrifuge tube. Centrifuge at 12,000×g for 1 minute and discard the supernatant.
 - (b) Add 100 µl of LB14 and 20 µl of Proteinase K to the cell pellets. Mix thoroughly to resuspend the cells.
If RNA removal is needed, add 20 µl of RNase A to the sample, incubate at room temperature for 2 minutes.
- Anti-coagulant Blood
 - (a) Add ≤50 µl of blood into a microcentrifuge tube.
 - (b) Add LB14 to a final volume of 100 µl, and add 20 µl of Proteinase K into the tube. Mix thoroughly by vortexing.
For fresh anti-coagulant blood, if RNA removal is needed, add 20 µl of RNase A to the sample, incubate at room temperature for 2 minutes (frozen blood is RNA-free).
- Serum/Plasma
 - (a) Add 50 µl-250 µl of serum/plasma into a 1.5 ml microcentrifuge tube.
 - (b) Optional: If the volume of serum/plasma is less than 100 µl, please add LB14 to a final volume of 100 µl.
 - (c) Add 20 µl of Proteinase K into the tube. Mix thoroughly by vortexing.
- Mouthwash
 - (a) Add 2-20 ml of mouthwash into a 50 ml sterilized tube. Centrifuge at 800×g for 5 minutes and discard the supernatant.
 - (b) Add 100 µl of LB14 to resuspend the pellets and transfer all suspension solution into a 1.5 ml microcentrifuge tube.
 - (c) Add 20 µl of Proteinase K into the tube, mix by vortex and incubate at room temperature for 2 minutes.
If RNA removal is needed, add 20 µl of RNase A to the sample, incubate at room temperature for 2 minutes.
- Dried Blood Spots
 - (a) Cut 5 mm²-100 mm² sample from a dried blood spot into small pieces and place it into a 1.5 ml microcentrifuge tube.
 - (b) Add 100 µl LB14 and 20 µl Proteinase K into the tube, mix by vortex and incubate at room temperature for 2 minutes.
- 2. Add 500 µl of BB14 and 1 µl of Carrier RNA. Mix thoroughly by vortex and incubate at 55°C in water bath for 10 minutes.
Vortex twice during the incubation.
- 3. Apply all the mixture to a spin column, centrifuge at 12,000×g for 30 seconds, discard the flow-through.
- 4. Add 500 µl of CB14, centrifuge at 12,000×g for 30 seconds, and discard the flow-through.
- 5. Add 500 µl of WB14 (check to ensure you have added ethanol before use), 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 remaining WB14.



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8. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 30-100 μ l of Elution Buffer (preheated to 65°C) or sterile, distilled water (pH >7.0) to the center of column. Incubate at room temperature for 1 minute. Centrifuge at 12,000 \times g for 1 minute to elute the isolated DNA.
9. To recover more DNA, repeat step 8 once. Store the isolated DNA at -20°C.

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