EasyPure® Micro Genomic DNA Kit

Cat. No. EE181

Storage: 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, microtissue, microdissected samples and formaldehyde fixed tissue. 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 down-stream applications.

Features:

1.Fast

2.High yield and purity

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
E. coli	≤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 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 Samples, Formaldehyde Fixed Samples 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 mm^2-100 mm^2 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 three 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.

8. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 30-100 µl 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×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.

Notes

1.The sample volume should not be too much or too small, so as not to affect the extraction effect.

2.Try to chop the tissue as much as possible so as not to affect the lysis effect; after complete lysis, the solution is viscous and non-gel-like.

3.In order to ensure the quality of the extracted DNA, use fresh materials to avoid repeated freeze-thaw; the quality of DNA depends on the type of material, storage method, time, etc.

4.Use sterile centrifuge tubes and tips to avoid DNase contamination.

5.The second elution is recommended to use the first elution liquid or add a smaller amount of eluent than the first to avoid affecting the extraction concentration.

6.Micro samples have yielded fewer genomes, especially serum/plasma, etc., so the genomes obtained from these samples are not recommended for agarose electrophoresis and spectrophotometer detection.

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