Genomic DNA Purification Kit

Catalog No.: B0007

Description

The EZBioscience® Genomic DNA Purification Kit provides a simple, reliable and rapid method for isolation of genomic DNA from cultured cells and animal tissues.

The kit is based on the highly efficient and reversible adsorption of DNA by silica membrane. High molecular weight genomic DNA purified with the kit is suitable for direct use in all common molecular biology applications: PCR, cloning, DNA sequencing, digestion with restriction endonucleases, and membrane hybridization (e.g., Southern blot analysis).

The biological samples are first lysed and homogenized in a strong denaturant containing buffer, which immediately generates an aqueous solution containing the cellular components. The aqueous solution is then applied to the column to bind the genomic DNA. Any impurities are effectively removed by subsequent washing with Wash Buffer 1 and Wash Buffer 2. Finally, the purified genomic DNA is eluted with the Elution Buffer, which can be used in a variety of downstream applications.

Components

Component	B0007 (100 Preps)
Lysis Buffer	60 ml
Wash Buffer 1[a]	24 ml
Wash Buffer 2[b]	12 ml
Elution Buffer	25 ml
Spin Columns (with Collection Tubes)	100 preps

[a] Before using for the first time, add 36 ml of 100% ethanol to the Wash Buffer 1.

[b] Before using for the first time, add 48 ml of 100% ethanol to the Wash Buffer 2.

Storage

Divide the Elution Buffer into small aliquots upon reception and store at -20°C. Store other co mponents at room temperature (When using these buffers, be careful to avoid of contamination).

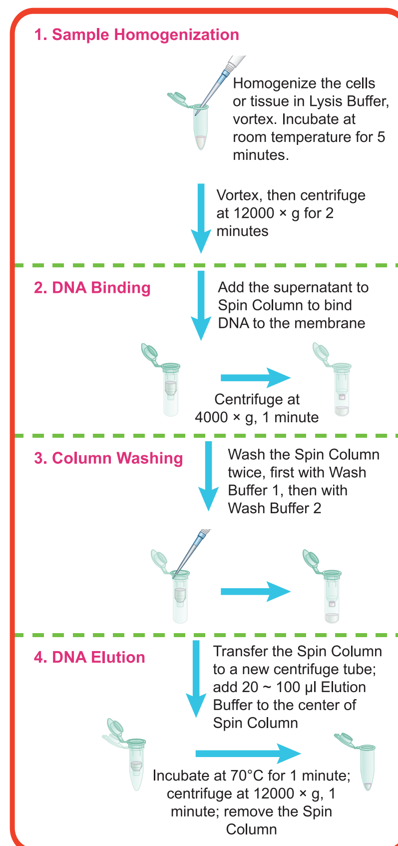
Protocol

Sample Homogenization

1A. For adherent cells ≤ 3 × 10^6/sample:

a) Remove the growth medium from the cells.

Experimental Procedure Overview:



b) Wash cells with appropriate volume of PBS.

c) Add 500 µl of Lysis Buffer. Pipette up and down for 10 times to suspend the cells.

d) Transfer the cell lysate to a new tube and vortex at high speed for 10 seconds until the cells appear completely lysed.

1B. For cultured suspension cells or adherent cells more than 3 × 10^6/sample:

a) (*For adherent cells only, for suspension cells, start at step b*) Detach cells using the subculturing method routinely employed in your laboratory.

b) Pellet 1 × 10^6 cells in a 1.5 ml centrifuge tube by centrifugation at 500 × g for 1 minute.

c) Completely remove the supernatant by aspiration.

d) Add 500 µl of Lysis Buffer.

e) Vortex at high speed for 10 seconds until the cell pellet is completely dispersed and the cells appear lysed.

1C. For animal tissues:

a) Place 1 ~ 100 mg tissue in a 1.5 ml centrifuge tube. Add 300 µl of Lysis Buffer.

b) Homogenize the tissue with a pestle or rotor-stator homogenizer. In case the tissue is larger than 5 mg, transfer a volume of the homogenate that contains 5 mg tissue to a new tube.

c) Add Lysis Buffer to the homogenate to a total volume of 300 μl. Homogenize again, then vortex for 10 seconds at high speed to lyse the tissue completely.

2. Incubate the cell or tissue lysate at room temperature for 5 minutes, then vortex for 10 seconds.

3. Centrifuge at 12000 × g for 2 minutes.

DNA Binding

4. Transfer the supernatant to the Spin Column.

5. Centrifuge at 4000 × g for 1 minute (for tissue sample, 12000 × g is recommended).

Column Washing

6. Add 500 μl of Wash Buffer 1 to the column. Centrifuge at 12000 × g for 1 minute.

7. Add 500 μl of Wash Buffer 2 to the column. Centrifuge at 12000 × g for 1 minute.

8. Transfer the column to a new 1.5 ml centrifuge tube. Keep the lid open to air-dry the column for 2 minutes at room temperature.

DNA Elution

9. Add 20 ~ 100 μl of Elution Buffer to the center of the column, and incubate at 70°C for 1 minute.

10. Centrifuge at 12000 × g for 1 minute.

11. Discard the column，determine the DNA concentration, do the following experiment with the purified DNA, or store the DNA at -80°C until needed.

Representative Results

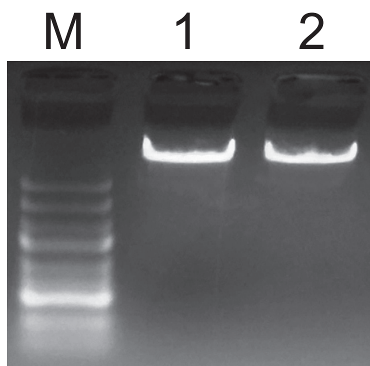


Figure 1. Genomic DNA isolated from 5 mg mouse kidney tissue (two samples) using EZBioscience® Genomic DNA Purification Kit. DNA was eluted by 50 μl Elution Buffer, and 5 μl was loaded each lane. M: 250bp DNA Ladder; Lane 1: DNA isolated from 5 mg kidney tissue, No. 1; Lane 2: DNA isolated from 5 mg kidney tissue, No. 2.

The data above shows that, the EZBioscience® Genomic DNA Purification Kit can isolate genomic DNA from animal tissues efficiently.

Trouble shooting

The yield of DNA is too low.

Suggestions:

a. Examine whether any of the reagents in the Kit is contaminated: use a new Kit as positive control to confirm whether the reagents are contaminated (If the reagents are contaminated, the results between these two will be different, obviously). Therefore, it is recommended to divide the reagents into 3 ~ 4 parts, each part in a 15 ml/50 ml centrifuge tube. Be careful when taking the reagents out of the bottles to avoid of contamination.

b. Increase the amount of sample used.

c. Examine whether the experiment is carried out correctly.

e.g.:

1. During the whole process of DNA purification by this Kit, it must be manipulated at room temperature but not on ice, to avoid of blocking the membrane in the Spin Column by the water-insoluble substances formed during ice cooling.

2. Before using this Kit for the first time, add 36 ml 100% ethanol to the Wash Buffer1, add 48 ml 100% ethanol to Wash Buffer2, and mix thoroughly.

3. To get better results, wash the cells once with PBS before cell lysing is recommended.

4. The cells or tissues lysates should be mixed adequately by using vortex.

5. The columns should be washed first by Wash Buffer 1, then by Wash Buffer 2.

6. When washing the sample, the centrifuge should be set at 12000 × g, to eliminate the Wash Buffer adequately, and then open the lid and keep in the air for 2 minutes.

7. The volume of Elution Buffer can be adjusted from 20 μl to 100 μl, according to the DNA quantity predicted.