

Blood/Cell/Tissue Genomic DNA Extraction Kit

(Spin Column)

M665559

Introduction:

This kit is suitable for rapid and simple extraction of genomic DNA from various blood/cell/tissue samples. The extracted genomic DNA features large fragment size, high purity, and good stability, and can be directly used in experiments such as PCR, enzyme digestion, and hybridization. The extraction process does not require phenol-chloroform extraction: blood, cells, or tissues are lysed with lysis buffer and digested with Proteinase K, then can bind to Spin Columns GH4 under the adjustment of absolute ethanol. Residual impurities such as proteins and salts are removed through rapid and thorough washing, and finally, DNA is eluted in Buffer EB.

Product Components and Storage Conditions:

| M665559 | Component | 50T | Storage |
|----------|-------------------------|--------|---------|
| M665559A | Buffer DA | 15 ml | RT |
| M665559B | Buffer DLB | 15 ml | RT |
| M665559C | Buffer DW1 | 13 ml | RT |
| M665559D | Buffer DW2Plus | 12 ml | RT |
| M665559E | Buffer EB | 15 ml | RT |
| M665559F | Proteinase K (20 mg/ml) | 1.2 ml | RT |
| M665559G | Spin Columns GH4 | 50 pcs | RT |
| M665559H | 2 ml Collection Tubes | 50 pcs | RT |

Storage Conditions:

All components of this kit can be stored at room temperature (15°C-25°C) under dry conditions for 12 months. Before use, check if Buffer DA and Buffer DLB have crystals or precipitates. If crystals or precipitates are present, place Buffer DA and Buffer DLB in a 37°C water bath to redissolve.

Precautions:

1. Samples should avoid repeated freezing and thawing, as this will result in smaller extracted DNA fragments and reduced extraction yield.
2. Before first use, 17mL of absolute ethanol must be added to Buffer DW1 (13mL), and 48mL of absolute ethanol must be added to Buffer DW2Plus (12mL).
3. If RNA removal is required, RNase A (100 mg/ml) (Catalog No.: R665518) must be prepared separately.
4. Absolute ethanol must be prepared separately.

Operating Steps:

Before first use, 17mL of absolute ethanol must be added to Buffer DW1 (13mL), and 48mL of absolute ethanol must be added to Buffer DW2Plus (12mL).

1. Sample Processing:
 - 1.1 Mammalian blood: Take 200µl of fresh or anticoagulated blood into a 1.5 ml centrifuge tube, and proceed directly to steps 2-10. If the blood volume is less than 200µl, make up to 200µl with Buffer DA.
 - 1.2 Avian, poultry, and amphibian blood: Take 5-20 µl of fresh or anticoagulated blood into a 1.5 ml centrifuge tube, and make up to 200µl with Buffer DA.
 - 1.3 Tissue culture cells: Collect approximately 10^5 - 10^6 suspension cells (total cell count should not exceed 5×10^6) into a 1.5ml centrifuge tube; for adherent cells, first digest with trypsin and resuspend by pipetting. Centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 1min, discard the supernatant, add 200µl Buffer DA to the cell pellet, and vortex until completely resuspended.
 - 1.4 Animal tissue: Weigh < 25 mg of animal tissue (spleen should be less than 10 mg), grind into fine powder with liquid nitrogen or cut into small pieces with a scalpel, and transfer to a 1.5 ml centrifuge tube pre-filled with 180µl Buffer DA. In addition to efficiently extracting DNA from blood, cells, and tissues, this kit can also extract high-quality DNA from bacteria.
 - 1.5 Bacteria: Take 1-5 ml of bacterial culture, centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 1 min, and aspirate the supernatant as much as possible.
 - (1) For gram-positive bacteria with difficult cell wall lysis, add lysozyme solution for cell wall disruption: Add 110µl buffer (20 mM Tris, pH 8.0; 2 mM $\text{Na}_2\text{-EDTA}$; 1.2% Triton) and 70µl lysozyme solution (50 mg/ml, provided by the user), and incubate at 37°C for at least 30 min.
 - (2) For relatively easily lysed bacteria, directly add 200µl Buffer DA to the bacterial pellet and vortex until the bacteria are completely resuspended.
2. Optional step: If RNA removal is required (for cells, tissues, and bacteria), add 4µl RNase A (100 mg/ml) solution (Catalog No.: R665518). Vortex for 15 sec and incubate at room temperature for 5 min.
3. Add 20µl Proteinase K and vortex to mix thoroughly.
 - 3.1 For blood, cell, or bacterial samples, simply add Proteinase K, mix well, and proceed to the next step.

- 3.2 For animal tissue samples, add Proteinase K and vortex to mix. Incubate at 56°C until the tissue is completely dissolved, then briefly centrifuge to collect the solution on the inner wall of the tube cap before proceeding to the next step.
- Note: Lysis time varies for different tissues, usually taking 1-3 hours (mouse tail requires 6-8 hours of digestion, and overnight digestion is necessary if needed), which will not affect subsequent operations.
4. Add 200µl Buffer DLB, invert thoroughly to mix, incubate at 70°C for 10 min, and briefly centrifuge to collect the solution on the inner wall of the tube cap.
- Note: White precipitate may form when adding Buffer DLB, but it usually disappears during incubation at 70°C and will not affect subsequent experiments.
5. Add 200µl absolute ethanol and mix (precipitate may appear at this point). Transfer the resulting solution and precipitate to the adsorption column Spin Columns GH4 (place the column in the collection tube), centrifuge at 12,000 rpm (~13,400×g) for 30 sec, discard the filtrate, and return the adsorption column to the collection tube.
6. Add 500µl Buffer DW1 (ensure absolute ethanol is added before use) to the adsorption column, centrifuge at 12,000 rpm (~13,400×g) for 30 sec, discard the waste liquid, and return the adsorption column to the collection tube.
7. Add 500µl Buffer DW2Plus (ensure absolute ethanol is added before use) to the adsorption column, centrifuge at 12,000 rpm (~13,400×g) for 30 sec, discard the waste liquid, and return the adsorption column to the collection tube.
8. Repeat step 7 once.
9. Centrifuge at 12,000 rpm for 3 min, and discard the waste liquid in the collection tube.
10. Transfer the adsorption column into a new 1.5 ml centrifuge tube, then add 60-200µl of Buffer EB to the central part of the adsorption membrane while avoiding contact. Incubate at room temperature for 1 min, centrifuge at 12,000 rpm (~13,400×g) for 1 min, and collect the DNA solution.
- Note: The volume of Buffer EB should not be less than 60µl, as a smaller volume will affect recovery efficiency. If water is used as the eluent, ensure its pH is within the range of 7.0-8.5 (NaOH can be used to adjust the pH of water to this range). Store the DNA solution at -20°C.

Frequently Asked Questions and Solutions:

| Frequently Asked Questions | Cause | Solution |
|----------------------------|--|--|
| Column blockage | 1. Too much sample input | Please use samples within the compatible range. |
| | 2. Inadequate Sample Lysis | Extend the 56°C water bath time and increase the number of inversion and mixing times. |
| Low DNA Yield | 1. The sample has been repeatedly frozen and thawed | Avoid repeated freezing and thawing of samples. It is recommended to use fresh samples or samples that have been frozen once and thawed only once. |
| | 2. Inadequate lysis of animal tissue | Cut tissue blocks into small pieces as much as possible or grind them with liquid nitrogen. During extraction, ensure that the samples are fully mixed with Buffer DA and Proteinase K in a timely manner, and the 56°C water bath lysis time can be appropriately extended. |
| | 3. Incomplete transfer of the lysis mixture into the adsorption column | After cell lysis, add absolute ethanol, flocculent precipitates will appear in the solution, which need to be transferred to the adsorption column together with the solution. |
| | 4. Issues with the eluent | Please use the Buffer EB provided with the kit for elution. If using ddH ₂ O or other eluents, ensure that the pH value of the eluent is between 7.0 - 8.0. Buffer EB can be preheated to 55°C in advance before elution, which is beneficial to improve the DNA yield. |
| | 5. Low elution efficiency | The eluent should be dropped in the center of the membrane; increase the elution volume or the number of elutions. |
| | 6. Failure to add absolute ethanol to Buffer DW1 / DW2 Plus | Add the specified volume of absolute ethanol to Buffer DW1 and Buffer DW2Plus as indicated on the bottle labels. |
| Low DNA Purity | 1. Contamination with foreign proteins | Buffer DW1 was not used for rinsing, or the correct volume of absolute ethanol was not added to Buffer DW1. Please add the specified volume of absolute ethanol according to the bottle label before operation. |
| | 2. Contamination with impurity ions | Buffer DW2Plus was not used for rinsing, or rinsed only once. Please rinse twice with Buffer DW2Plus according to the instruction manual. |