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Fast Magbead Tissue DNA Kit

Catalog No.	Specification	Storage/Shelf life
EP026	96T	Room temperature/1 year

Introduction

This kit provides a simple, rapid and efficient method for extracting DNA from fresh or frozen tissues. After tissue lysis, the DNA binds to the surface of silica-coated magnetic beads. After rinsing, the high-purity DNA is eluted in buffer TB or deionized water. The purified DNA can be directly used in PCR、Real-time PCR、SNP、STR 、next-generation sequencing, pharmacogenomics research and so on. Besides being able to rapidly extract DNA from animal tissues, this kit is also applicable to the extraction of DNA from samples such as blood, cells and bacteria.

Storage Condition：All components can be stably stored in a dry environment at room temperature (15 - 30℃).

Components

Component	EP026 96 Preps
Buffer ATL	25 mL
Buffer AL	36 mL
Buffer MB	70 mL
Buffer DW	70 mL
Buffer TB	15 mL
Proteinase K（20 mg/mL）	2×1.25 mL
RNase A（100 mg/mL）	0.6 mL
Magbeads PN	4×1.25 mL

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Self-prepared instruments and reagents

1. Kangwei CWE2100 and CWE960 Fully Automatic Nucleic Acid Extractors
2. Absolute ethanol
3. 96 DW Plate, 8 channel Comb

Preparations before the Experiment and Important Precautions

1. Please check whether Buffer ATL and Buffer AL have crystals or precipitates before use. If there are crystals or precipitates, please redissolve Buffer ATL and Buffer AL in a water bath at 56°C.
2. Before starting the experiment, preheat the water bath or constant temperature mixer to 56°C.
3. Magbeads PN must not be frozen or subjected to high-speed centrifugation, otherwise it may cause irreversible damage to Magbeads PN. Please shake Magbeads PN thoroughly to ensure uniform mixing each time it is used.

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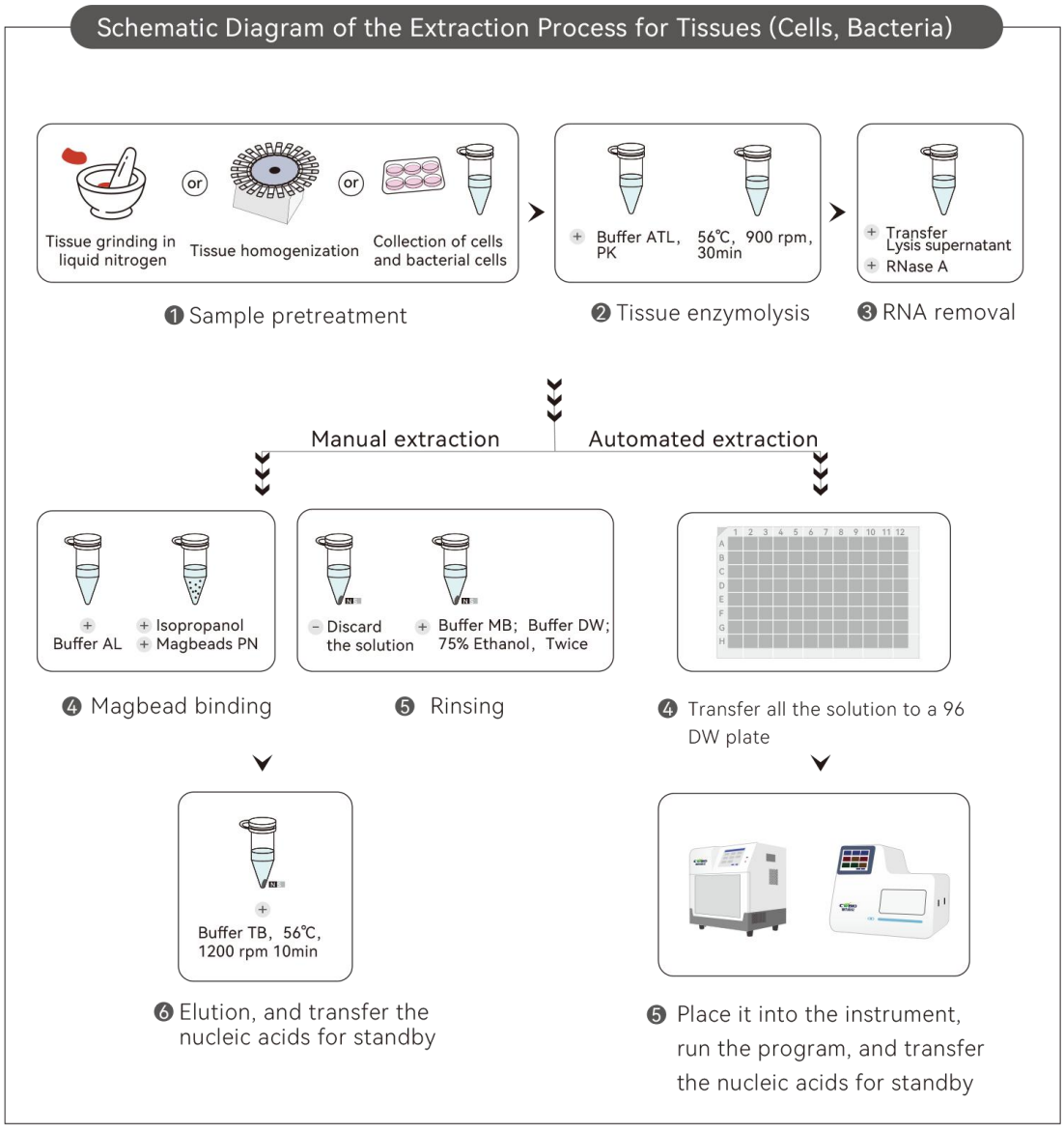
Operating procedures



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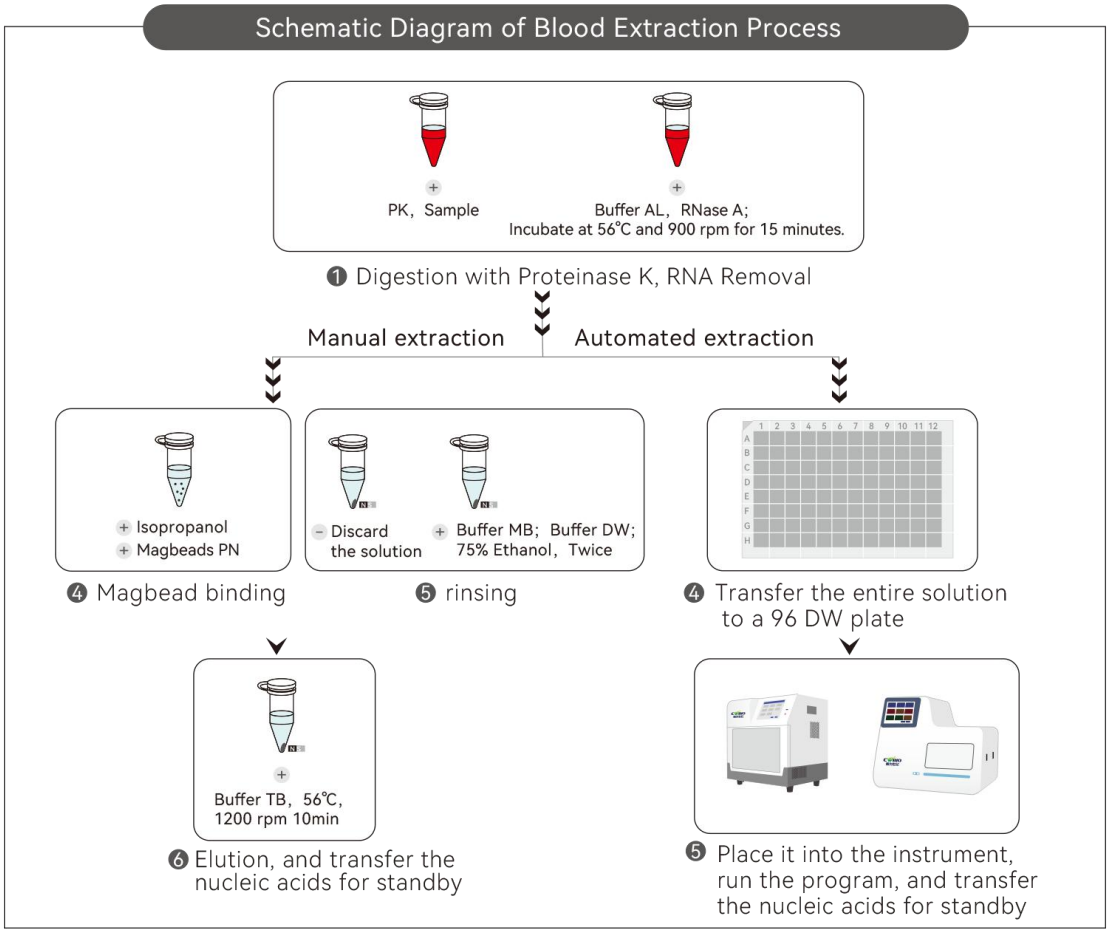

Manual operation steps (for animal tissues)

This protocol is applicable to both fresh and frozen tissue samples. The yield and quality of the purified DNA depend on the tissue type, source, and storage conditions. (After the samples such as cells and bacteria obtain the precipitate according to the recommended loading amount, the extraction operation for animal tissues can be carried out. Gram-positive bacteria need to be pretreated with lysozyme.)

1. Weigh 25 - 30 mg (the amount used for the spleen should be less than 10 mg) of animal tissue. Grind the tissue into fine powder using liquid nitrogen or cut it into small pieces with a scalpel. Transfer the tissue into a 2 mL centrifuge tube with 220 μL of Buffer ATL pre-added, mix thoroughly by vortexing, and then centrifuge instantaneously.

2. Add 20 μL of Proteinase K, mix thoroughly by vortexing again, and centrifuge

instantaneously.

3. Incubate at 56°C with a rotation speed of 900 rpm for 30 minutes until the tissue is completely dissolved. Centrifuge briefly to collect the solution on the inner wall of the tube cap.

4. Centrifuge at 14000 g for 2 minutes, and then aspirate 200μL of the lysate from the previous step into a new 2 mL centrifuge tube.

5. Add 5 μL of RNase A, gently vortex to mix evenly, and let stand at room temperature for 10 minutes.

6. Add 150 μL of Buffer AL, gently vortex to mix evenly, and centrifuge instantaneously.

7. Add 280μL of isopropanol and 40μL of Magbeads PN, gently vortex to mix evenly for 10 seconds, centrifuge instantaneously, then shake and mix evenly at 1200 rpm for 10 minutes at room temperature. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube.

8. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★ until the solution becomes clear). Aspirate and discard the solution, avoiding touching the magnetic beads during the process.

9. Add 650 μL of Buffer MB to the centrifuge tube, gently vortex to mix evenly for 10 seconds to resuspend the magnetic beads, then shake and mix evenly at 1200 rpm for 3 minutes at room temperature. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★until the solution becomes clear). Aspirate and discard the solution,avoiding touching the magnetic beads during the process.

10. Add 650μL of Buffer DW to the centrifuge tube, gently vortex to mix evenly for 10 seconds to resuspend the magnetic beads, then shake and mix evenly at 1200 rpm for 3 minutes at room temperature. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★until the solution becomes clear). Aspirate and discard the solution,avoiding touching the magnetic beads during the process.

11. Add 650μL of 75% ethanol (prepared by yourself), gently vortex to mix evenly for

10 seconds to resuspend the magnetic beads, then shake and mix evenly at 1200 rpm for 3 minutes at room temperature. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★until the solution becomes clear). Aspirate and discard the solution,avoiding touching the magnetic beads during the process.

12. Repeat step 11 once.

13. Let the centrifuge tube with the supernatant completely removed dry at room temperature for about 3-5 minutes until the surface of the magnetic beads turns dull black and matte. Add 80-100 μL of Buffer TB, gently vortex to mix evenly for 10 seconds to resuspend the magnetic beads, then incubate at 56°C and 1200 rpm for 10 minutes. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube. Transfer to a magnetic rack and let it adsorb for 1 minute, then aspirate the supernatant into a new centrifuge tube for standby. The purified DNA can be stored at –20°C for long-term standby.

Manual operation steps (for mammalian blood)

This protocol is applicable to frozen or fresh anticoagulated whole blood (EDTA, sodium citrate) samples. For frozen blood samples, they need to be quickly thawed at 37°C and shaken appropriately to mix thoroughly and ensure that the samples are equilibrated to room temperature before starting the operation. The yield and quality of the purified DNA depend on the storage conditions of the blood, and fresh blood samples may yield better results.

1. In a 1.5 mL centrifuge tube, add 20 μL of Proteinase K and 200μL of blood, and gently vortex to mix evenly.

2. Centrifuge instantaneously. Then add 300 μL of Buffer AL and 5μL of RNase A, gently vortex to mix evenly for 10 seconds, centrifuge instantaneously, and incubate at 56°C and 900 rpm for 15 minutes.

3. After the incubation is completed, centrifuge instantaneously. Then add 300μL of isopropanoland 20μL of Magbeads PN, gently vortex to mix evenly for 10 seconds, shake and mix evenly at 900 rpm for 5 minutes at room temperature. After that,

centrifuge instantaneously to ensure there is no liquid on the cap of the tube.

4. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★ until the solution becomes clear). Carefully aspirate and discard the solution, avoiding touching the magnetic beads during the process.

5. Add 650 μL of Buffer MB to the centrifuge tube, gently vortex to mix evenly for 10 seconds to resuspend the magnetic beads, then shake and mix evenly at 1200 rpm for 3 minutes at room temperature. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★until the solution becomes clear). Aspirate and discard the solution, avoiding touching the magnetic beads during the process.

6. Add 650 μL of Buffer DW to the centrifuge tube, gently vortex to mix evenly for 10 seconds to resuspend the magnetic beads, then shake and mix evenly at 1200 rpm for 3 minutes at room temperature. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★until the solution becomes clear). Aspirate and discard the solution,avoiding touching the magnetic beads during the process.

7. Add 650 μL of 75% ethanol (prepared by yourself) to the centrifuge tube, gently vortex to mix evenly for 10 seconds to resuspend the magnetic beads, then shake and mix evenly at 1200 rpm for 3 minutes at room temperature. After that, centrifuge instantaneously to ensure there is no liquid on the the cap of the tube. Transfer the centrifuge tube to a magnetic rack and let it adsorb for 1 minute (★until the solution becomes clear). Aspirate and discard the solution, avoiding touching the magnetic beads during the process.

8. Repeat step 7 once.

9. Let the centrifuge tube with the supernatant completely removed dry at room temperature for about 3-5 minutes until the surface of the magnetic beads turns dull black and matte. Add 80-100 μL of Buffer TB, gently vortex to mix evenly for 10 seconds to resuspend the magnetic beads, then incubate at 56°C and 1200 rpm for 10 minutes. After that, centrifuge instantaneously to ensure there is no liquid on the cap of the tube. Transfer to a magnetic rack and let it adsorb for 1 minute, then aspirate the supernatant into a new centrifuge tube for standby. The purified DNA

can be stored at -20°C for long-term standby.

Automated extraction (for animal tissues)

(After obtaining the precipitate according to the recommended loading amount for samples such as cells and bacteria, the extraction operation for animal tissues can be carried out. Gram-positive bacteria need to be pretreated with lysozyme.)

1. Weigh 25-30 mg (the amount used for the spleen should be less than 10mg) of animal tissue. Grind it into a fine powder using liquid nitrogen or cut it into small pieces with a scalpel. Transfer it into a 2 mL centrifuge tube pre-filled with 220μL of Buffer ATL, vortex thoroughly to mix well, and centrifuge instantaneously.
2. Add 20 μL of Proteinase K, vortex to mix evenly, and centrifuge instantaneously.
3. Incubate at 56°C and 900 rpm for 30 minutes until the tissue is completely dissolved. Centrifuge briefly to collect the solution on the inner wall of the tube cap.
4. Centrifuge at 14000g for 2 minutes. Aspirate 200μL of the lysate from the previous step into a new 2 mL centrifuge tube.
5. Add 5μL of RNase A (100 mg/mL) and let it stand at room temperature for 5 - 10 minutes. Transfer the entire solution to a 96-deep well plate. Add the remaining reagents according to the corresponding entries in the following table:

Position	Reagents and Their Dosages	Position	Reagents and Their Dosages
1&7 Colume	Lysis supernatant: All Buffer AL: 150 μL Isopropanol: 280 μL Magbeads PN: 40 μL	Plate 1	Lysis supernatant: All Buffer AL: 150 μL Isopropanol: 280 μL Magbeads PN: 40 μL
2&8 Colume	Buffer MB: 650 μL	Plate 2	Buffer MB: 650 μL
3&9 Colume	Buffer DW: 650 μL	Plate 3	Buffer DW: 650 μL
4&10 Colume	75% ethanol: 650 μL	Plate 4	75% ethanol: 650 μL
5&11 Colume	75% ethanol: 650 μL	Plate 5	75% ethanol: 650 μL

6&12 Colume	Buffer TB: 100 μL	Plate 6	Buffer TB: 100 μL

6. Place the 96-well deep well plate with the added reagents into the instrument in sequence, then insert the magnetic rod sleeve and run the program.
7. After the program is completed, take out the 96-well plate, transfer the nucleic acids into a 1.5 mL centrifuge tube, and store the product at -20°C.

Automated extraction (for mammalian blood)

1. Add 20 μL of Proteinase K and 200 μL of blood into a 1.5 mL centrifuge tube, and gently vortex to mix evenly.
2. Centrifuge instantaneously. Then add 300 μL of Buffer AL and 5 μL of RNase A, gently vortex to mix evenly for 10 seconds, centrifuge instantaneously, and incubate at 56°C and 900 rpm for 15 minutes.
3. After the incubation is completed, transfer the entire solution to a 96DW deep well plate. Add the remaining reagents according to the corresponding entries in the following table

Position	Reagents and Their Dosages	Position	Reagents and Their Dosages
1&7 Colume	Lysis supernatant: All Isopropanol: 300 μL Magbeads PN: 20 μL	Plate 1	Lysis supernatant: All Isopropanol: 300 μL Magbeads PN: 20 μL
2&8 Colume	Buffer MB: 650 μL	Plate 2	Buffer MB: 650 μL
3&9 Colume	Buffer DW: 650 μL	Plate 3	Buffer DW: 650 μL
4&10 Colume	75% ethanol: 650 μL	Plate 4	75% ethanol: 650 μL
5&11 Colume	75% ethanol: 650 μL	Plate 5	75% ethanol: 650 μL
6&12 Colume	Buffer TB: 100 μL	Plate 6	Buffer TB: 100 μL

4. Place the 96-well deep well plate with the added reagents into the instrument in sequence, then insert the magnetic rod sleeve and run the program.
5. After the program is completed, take out the 96-well plate, transfer the nucleic acids into a 1.5 mL centrifuge tube, and store the product at -20°C.

Result analysis

The DNA concentration should be determined by measuring the absorbance at 260 nm (A260) with a spectrophotometer. DNA has a significant absorption peak at 260 nm, and the absorbance reading should be between 0.1 and 1.0. If it is not within this range, the sample needs to be diluted or concentrated. At 260 nm, one unit of absorbance corresponds to 50 μg of DNA per milliliter (A260= 1 → 50 μg/mL). Residual magnetic beads in the eluent may affect the A260 reading but will not affect the performance of the downstream DNA.

DNA sample concentration = 50 μg/mL × (A260- A320) × dilution factor Total amount of DNA = concentration × sample volume (mL)

The DNA purity is determined by calculating the ratio of the corrected absorbance at 260 nm to that at 280 nm (subtracting the absorbance reading obtained at 320 nm to correct for the presence of magnetic particles), that is, (A260 – A320)/(A280 – A320). The A260/A280 ratio of pure DNA is 1.7 - 1.9.