AFTSpin Blood/Tissue/Cell Fast DNA Extraction Kit

Cat. No. : RK30110

Product components

Components	Component number	Size 50 RXN	Storage
Proteinase K (20 mg/mL)	RM30130	1.2 mL	RT
Buffer DA	RM30131	15 mL	RT
Buffer DLB	RM30132	15 mL	RT
Buffer DW1	RM30133	13 mL	RT
Buffer DW2 Plus	RM30134	12 mL	RT
Buffer EB2	RM30153	15 mL	RT
Spin Column 4	RM30187	50 pcs	RT
2 mL Collection Tubes	RM30188	50 pcs	RT

Product Description

This kit is suitable for quick and simple extraction of genomic DNA from various samples inclouding blood, cells and tissue. The extracted genomic DNA fragment are large, high purity and good stability, and can be directly used in PCR, enzyme digestion and hybridization experiments. The extraction process does not require phenol-chloroform extraction. Blood, cells or tissues are lysed by lysate and digested by Proteinase K, and then can be combined with Spin Column 4 under the regulation of anhydrous ethanol. After quickly and sufficiently washing, residual impurities such as protein and salt are removed, and finally DNA is dissolved in Buffer EB2.

Storage Conditions

Reagents are stable for 12 months at room temperature. Before use, please check whether the Buffer DA and Buffer DLB have crystallization or precipitation. If there is crystallization or precipitation, please put the Buffer DA and Buffer DLB in a 37℃ water bath to dissolve again.

Highlights

1. Universal extraction of genomic DNA from animal tissues, blood and cell samples.
2. Operation is simple and convenient, and the extraction time for a single tube does not exceed 20 minutes.
3. The extracted nucleic acid has high quality and purity, and can be applied to various downstream applications such as PCR, enzyme digestion, and chip detection.

Operational Instructions

Materials to be provided by the user

1. Absolute ethanol
2. 1.5 mL sterile centrifuge tubes

Reagent Preparation

Before the first use, please add 17 mL of absolute ethanol into Buffer DW1 bottle and 48 mL of absolute ethanol into Buffer DW2 Plus.

Precautions

1. The sample should avoid repeated freeze-thaw, otherwise the extracted DNA fragments will be smaller and the extracted amount will also decrease.
2. If RNA needs to be removed, the reagent RNase A (100 mg/mL) should be prepared.
3. Prepare absolute ethanol reagent.

Operation steps

1. Sample processing
   a. **Mammalian blood**: 200 μL of fresh or anticoagulated blood is put into a 1.5 mL centrifuge tube, and can proceed the next steps 2-10. If the blood is less than 200 μL , adjust the blood sample volume to 200 μL by adding Buffer DA.
   b. **Blood of poulty, birds and amphibians**: 5-20 μL fresh or anticoagulant into a 1.5 mL centrifuge tube and adjust the blood sample volume to 200 μL by adding Buffer DA.
   c. **Cultured cells**: collect about 10^5-10^6 suspended cells (the total number of cells should not exceed 5 × 10^6) to a 1.5 mL centrifuge tube. For adherent cells, trypsinization and pipetting should be used firstly, and then centrifuge at 12,000 rpm (~13,400 × g) for 1 min, discard the supernatant and add 200 μL Buffer DA to the cell pellet,and shake to completely resuspend.
   d. **Animal tissue:** weigh < 25 mg (spleen dosage should be less than 10 mg) of animal tissue, grind it into fine powder by liquid nitrogen or cut it into small pieces with a scalpel, and transfer it into a 1.5 mL centrifuge tube pre-filled 180 μL Buffer DA.
   e. **Bacteria:** take 1-5 mL of bacterial culture solution, centrifuge at 12,000 rpm (~13,400 × g) for 1 min and try to absorb the supernatant.
      For gram-positive bacteria which are difficulty to break the wall, add lysozyme solution to break the wall. The specific method is: add 110 μL buffer (20 mM Tris, pH 8.0; 2 mM Na_2-EDTA; 1.2% Triton) and 70 μL lysozyme solution (50 mg/mL, prepared by the customer), treated at 37℃ for more than 30 minutes.
      For bacteria that are relatively easy to lysis, 200 μL Buffer DA can be directly added to the bacterial precipitation , shake it until the pellet is completely resuspended.
2. **Optional steps**: if RNA-free genomic DNA is required (cells, tissues and bacteria need to remove RNA), add 4 μL RNase A (100 mg/mL) solution. Shake it for 15 sec and place at room temperature for 5 min.
3. Add 20 μL Proteinase K, vortex oscillation mixing.
   a. If the extracted sample is blood, cells or bacteria, just add Proteinase K and mix it well, then go to the next step.
   b. If the extracted sample is animal tissue, add Proteinase K and mix it with vortex oscillation. Incubate it at 56℃until the tissue is completely dissolved. Centrifuge briefly to collect the solution on the inner wall of the tube cap, and then go to the next step.

   Note: For different tissues, the lysis time is different, it usually takes 1-3 hours to complete (rat tail needs to digest for 6-8 hours, and digest overnight if necessary), and it will not affect subsequent operations.
4. Add 200 μL Buffer DLB, fully inverted and mixed, incubated at 70℃ for 10 min, and centrifuged briefly to collect the solution on the inner wall of the tube cap.

   Note: When adding Buffer DLB, white precipitates may be produced, which will generally disappear when incubated at 70℃, and will not affect subsequent experiments.
5. Add 200 μL absolute ethanol, mix well (precipitation may occur at this time), transfer the obtained solution and precipitation together into the Spin Column 4 (the adsorption column is placed in the collection tube), centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds, discard the filtrate, and put the adsorption column into the collection tube.
6. Add 500 μL Buffer DW1 (add 17 mL of absolute ethanol into the bottle for the first time) to the adsorption column,centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds, discard the filtrate and put the adsorption column into the collection tube.
7. Add 500 μL Buffer DW2 Plus (add 48 mL of absolute ethanol into the bottle for the first time) to the adsorption column, centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds, discard the filtrate and put the adsorption column into the collection tube.
8. Repeat operation step 7 once.
9. Centrifuge at 12,000 rpm (~13,400 × g) for 3 min and discard the filtrate.
10. Transfer the adsorption column into a new 1.5 mL centrifuge tube, and add 60-200 μL Buffer EB2 to the center area of the

adsorption membrane. Stay at room temperature for 1 min, centrifuge at 12,000 rpm (~13,400 × g) for 1 min to obtain DNA solution.

Note: The volume of Buffer EB2 should not be less than 60 μL, or it will affect the recovery efficiency. If water is used as eluet, make sure its pH value should be within the range of 7.0-8.5 (NaOH can be used to adjust the pH value of water to this range). Please store DNA solution at -20 ℃.