ELK Biotechnology

For research use only.

Amp Blood Clot DNA Extraction Kit

Catalog No.	Specification	Storage/Shelf life
EP009-50T	50T	Room temperature/1 year
EP009-200T	200T	Room temperature/1 year

Introduction

The kit is suitable for extracting high purity total DNA from fresh or frozen animal tissues, cells, blood, bacteria and other samples. This product can be purified to obtain a DNA fragment with a molecular weight of up to 50 kb. The purification process does not require the use of toxic solvents such as phenol or chloroform, and ethanol precipitation is not required. The kit uses an optimized buffer system to efficiently and specifically bind the DNA in the lysate to a silica-based centrifugal adsorption column. The inhibitors of PCR and other enzymatic reactions can be effectively removed by a two-step washing step, and finally a low-salt buffer is used. High purity DNA can be obtained by eluting with liquid or water. Purified DNA can be directly used in downstream experiments such as digestion, PCR, Real-Time PCR, library construction, Southern hybridization and so on.

Kit Components

Component	EP009-50T	EP009-200T	Storage
Proteinase K	1 ml	4 ml	-20℃
Solution GAS1	25 ml	100 ml	RT
Solution GA2	25 ml	100 ml	RT
Wash Buffer	60 ml	240 ml	RT
Buffer RP	30 ml	120 ml	RT
Solution RCL	5 ml	10 ml	RT
Solution GE	10 ml	40 ml	RT
Adsorption column G column	50 sets	200 sets	RT
User manuals	1 copy	1 copy	RT

ELK Biotechnology

For research use only.

Optional reagent

RNase A（10 mg/ml）

Attention

1. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extraction amount will decrease.
2. If extracting the genome of a bacterial culture with a large accumulation of secondary metabolites or cell wall thickness, it is recommended to collect samples early in the logarithmic growth phase.
3. Add absolute ethanol to Wash Buffer and Solution RP as described in the label of the reagent bottle before the first use.
4. If Solution GAS1 and Solution GA2 are crystallized or precipitated, re-dissolve them in a 56 °C water bath, shake before use.
5. If downstream experiments are sensitive to RNA contamination, add 4μL of DNase-Free RNase A (100mg/ml) before adding Solution GA2. RNase A is not available in this kit and can be ordered separately from the company if needed.
6. All centrifugation steps are performed using a benchtop centrifuge and centrifuged at room temperature.

Operation steps

1. Processing blood clots (This kit is suitable for 100μl -1 ml blood clot samples.)

a. Take the blood clot into the EP tube, add 1-2.5 times the volume of cell lysate RCL, mix upside down, centrifuge at 12,000 rpm (≈11,500×g) for 1 min, discard the supernatant, and leave the nucleus pellet (if lysed Incomplete, you can repeat step b once);

b. Add 200μl GAS1 to the collected nucleus pellet, and shake to mix thoroughly.

Note: If RNA needs to be removed, add 4μlof RNase A (100 mg/ml) solution (customer-supplied), shake for 15 sec, and leave at room temperature for 5 min.

2. Add 20μl Proteinase K solution and mix well.

3. Add 300μl of buffer GA2, mix well by inversion, leave it at 56℃ for 10 min, mix it upside down several times, and clarify the solution strain (if the solution is not completely clarified, please extend the lysis time until the solution is clear).

4. Add 300μl of absolute ethanol (customer-supplied), mix thoroughly and invert, and flocculation may occur.

5. Add the solution obtained in the previous step and the flocculent precipitate to an adsorption column G (adsorption column G is placed in the collection tube), centrifuge at 12,000 rpm (≈ 11,500×g) for 30 sec, and drain the waste liquid in the collection tube to adsorb. Column G is placed in the collection tube

6. Add 500μl of Solution RP to the adsorption column G (check whether ethanol has been added before use), centrifuge at 12,000 rpm (≈11,500×g) for 30 sec, drain the waste liquid from the collection tube, and place the adsorption column G into the collection tube

7. Add 600 μ lWash Buffer to the adsorption column G (check whether ethanol has been added before use), centrifuge at 12,000 rpm (≈11,500×g) for 30 sec, drain the waste liquid from the collection tube, and place the adsorption column G into the collection tube

8. Repeat step 7

9. The adsorption column G was returned to the collection tube, centrifuged at 12,000 rpm (≈ 11,500 × g) for 2 min, and the waste liquid was discarded. The adsorption column G is allowed to stand at room temperature for several minutes to completely dry the residual ethanol remaining in the adsorbent material.

Note: The purpose of this step is to remove the residual ethanol in the adsorption column, and the residual ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.) experiment.

10. Transfer the adsorption column G into a 1.5 ml centrifuge tube, add 50-200μl (50μl recommended) TB to the middle of the adsorption membrane, place it at room temperature for 2-5 min, centrifuge at 12,000 rpm (≈11,500×g) for 2 min. The solution is collected into a centrifuge tube.

Note: The volume of the elution buffer should not be less than 50μl, and the volume is too small to affect the recovery efficiency. In order to increase the yield of genomic DNA, the centrifuged solution can be added to the adsorption column G column, left at room temperature for 2 min, and centrifuged at 12,000 rpm (~13,400×g) for 2 min. The pH of the eluent has a large effect on the elution efficiency. If ddH2O is used as the eluent, the pH should be within the range of 7.0-8.5. The pH below 7.0 will reduce the elution efficiency; and the DNA product should be stored at -20 ℃ to prevent DNA degradation.

DNA concentration and purity Detection

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shear force during operation. The recovered DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and ultraviolet spectrophotometry. DNA should have a significant absorption peak at OD260 with an OD260 value of 1 equivalent to approximately 50 μg/ml double-stranded DNA and 40 μg/ml single-stranded DNA. The ratio of OD260/OD280 should be 1.7–1.9. If the elution buffer is not used when eluting, and the deionized water is used, the ratio will be lower because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low

Frequently Questions & Answers

a,Column blocked

Suggestion: Please lyse the sample well, without obvious flocculation, proceed to the next step; wash with Solution RP multiple times (note that multiple washes will result in low genome recovery); disposable needle filter or 200-well screen filter

b, Low genome extraction rate

Suggestion:Increase digestion time, increase sample size, etc

c, Precipitate in solution is not dissolved

Suggestion: Solution will precipitate when the temperature is low. Please check if there is any precipitate before use. If there is precipitation, please incubate at 37 °C for a while, after the solution is clarified.

d, ethanol was not added to Wash Solution as required

Suggestion: Wash Solution does not add the required amount of ethanol according to the instructions to add the required amount of absolute ethanol, tighten the bottle cap after use to prevent ethanol volatilization

e,Selection of volume and time for dissolution

Suggestion: Dissolved volume will affect the final yield, the larger the dissolved volume, the higher the yield, but the concentration will be reduced. Please use the recommended volume of dissolution in the kit to ensure the best yield and concentration. After add Solution GE, 2~5 min at room temperature is more favorable for dissolving.