ELK Biotechnology

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Whole Blood DNA MiniPrep Kit

Catalog No.	Specification	Storage/Shelf life
EP008-50T	50T	Room temperature/1 year
EP008-200T	200T	Room temperature/1 year

Introduction

This product can rapidly separate and purify genomic DNA from 200-400 µl of fresh or frozen human or animal whole blood within 15-20 min. The product does not use proteinase K. After the whole blood is dissolved by Solution BFL1,the hemoglobin is removed by Solution BFL2 precipitation. The genomic DNA in the supernatant can be bound to the purification column. After being washed by Buffer RP and Wash Buffer, the remaining proteins on the membrane and the PCR inhibitors are removed,the genomic DNA is eluted by Elution Buffer and can be used in various molecular biology experiments immediately.

Kit Components

Component	EP008-50T	EP008-200T	Storage
Solution BFL1	20ml	80ml	RT
Solution BFL2	20ml	80ml	RT
Buffer RP	30ml	120ml	RT
Wash Buffer	60 ml	240 ml	RT
Elution Buffer	10 ml	40ml	RT
Adsorption column G column	50 set	200 set	RT
User manual	1 copy	1 copy	RT

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Before starting

Add absolute ethanol to Buffer RP and Wash Buffer as indicated on the label of the reagent bottle and tick the label in the circle of the label.

Note:

1. This procedure is designed to extract DNA from 400 µl of whole blood. If the blood volume is less than 400 µl but greater than 200 µl,the amount of Solution BFL1 and Solution BFL2 can be reduced proportionally (note that Solution BFL1 must be strictly followed: anticoagulated whole blood :Solution BFL2=3:4:3 volume ratio operation. otherwise it will lead to the next step can not be carried out). the other reagents do not change; if the blood volume is less than 200µl. it is recommended to supplement the blood with physiological saline to make the blood volume at least 200µl.
2. High fat blood and poultry blood may Not be applicable.

Operation steps

1. Add 300 µl of Solution BFL1 to a 1.5 ml centrifuge tube. Add 400 µl of anticoagulated whole blood,cover the tube,and vortex for 30 sec.

   Note: Do not add less than 400 µl volume of anticoagulated whole blood. If the blood volume is less than 400 µl,add normal saline to make the blood volume reach 400 µl.
2. Add 300 µl of Solution BFL2,shake the tube 3-5 times vigorously,and vortex for 30 sec.

   Note: This step will result in a large amount of hemoglobin precipitation.
3. Centrifuge at 12000 rpm (~13000×g) for 2 min at room temperature,More times

if necessary.

4. Carefully inhale the supernatant from step 3 into the column of the adsorption column G.**Do not absorb the lower layer of sediment, so as not to block the adsorption column and cause extraction failure!** Cover the tube,and centrifuge at 12000 rpm (~13000×g) for 60 sec at room temperature.

   Note: The volume of the supernatant obtained by centrifugation may be larger than that of the purified column due to the small amount of hemoglobin in the blood of some animals. At this time. it is recommended to draw 700 µl of the supernatant into the adsorption column G column. or to clear the supernatant. Perform the operation of this step twice.

5. Discard the filtrate. put the adsorption column G column back into the collection tube. add 500 µl Buffer RP to the adsorption column G column (check whether to add absolute ethanol before use). cover the tube. 12000 rpm (~ 13000 × g) Centrifuge for 60 sec.

   Note: If hemoglobin remains on the purified column membrane. it is normal and can be washed away by Buffer RP,Repeat this Step if necessary.

6. Discard the filtrate. put the adsorption column G column back into the waste collection tube. add 600 µl Wash Buffer to the adsorption column G column (check whether to add absolute ethanol before use). cover the tube cover. 12000 rpm (~ 13000×g) Centrifugation for 30 sec

7. Repeat Step 6.

8. Discard the filtrate. put the purification column back into the waste collection tube. and centrifuge at 12000 rpm (~13000×g) for 2 min at room temperature.
9. Transfer the adsorption column G column into a clean centrifuge tube. add 50-100µl Elution Buffer to the middle of the adsorption membrane. place it at room temperature for 2-5 minutes. centrifuge at 12.000 rpm (~13.400×g) for 1 min. Collect the solution into the centrifuge tube.
10. Note: 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 volume of the elution buffer should not be less than 100 µl. and the volume is too small to affect the recovery. The pH of the Elution Buffer has a large effect on the elution efficiency. If the water is used as the Elution Buffer. the pH should be within the range of 7.0-8.5 (the pH of the water can be adjusted to this range with NaOH). If the pH is lower than 7.0. the elution efficiency will be lowered; and the DNA product should be stored at -20. °C to prevent DNA degradation.

DNA concentration and purity etection

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

Attention

1. Samples should be protected from repeated freezing and thawing. otherwise the extracted DNA fragments will be smaller and the amount of extraction will be reduced.
2. If there is a precipitate in Solution BFL1 or Solution BFL2. re-dissolve in a 56 °C water bath. shake and use.
3. All centrifugation steps are performed using a benchtop centrifuge and centrifuged at room temperature.

Frequently Questions & Answers

1. genome or genome concentration was low

a. Column blocked
Suggestion: Try to use fresh blood samples. Multiple cleaning with SolutionRP (multiple cleaning will result in low genomic recovery). some frozen samples may have hemolysis and stagnation. and the centrifugation time can be increased to 10 min in the fourth step.
b. Low genome extraction rate
Suggestion: There are not many genomes in 200 µl blood. It is normal for the low concentration of running glue. If the subsequent demand is large. it can be extracted and concentrated after multiple extractions.
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. Wash Solution was not added to ethanol 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 Elution Buffer. 2~5 min at room temperature is more favorable for dissolving.