

Blood Genome Column Medium Extraction Kit (1–5 ml)

Project number: B669892

Storage conditions: 2–8° C.

Products

individual parts making up a compound	50T
BufferRCL	3×260ml
BufferGR	25ml
BufferGL	25ml
BufferGW1 (concentrate)	13ml
BufferGW2 (concentrate)	15ml
BufferGE	15ml
ProteinaseK	50mg
ProteinaseKStorageBuffer	5ml
SpinColumnsDLwithCollectionTubes	50

Products

This kit is suitable for the extraction of total DNA, including genomic DNA, mitochondrial DNA and viral DNA, from fresh or frozen whole blood (blood samples treated with anticoagulants such as citrate, EDTA or heparin), plasma, serum, haematocrit brown and yellow layers, bone marrow, cell-free body fluids, etc. The product can process 1–5 ml of whole blood, and can be purified to obtain sizes ranging from 100bp to 50kb. The purified DNA is of high yield and good quality, with maximum removal of proteins, pigments, lipids and other inhibitory impurities, and can be directly used in PCR, fluorescence quantitative PCR, enzyme digestion and Southern Blot.

Self-contained reagent: anhydrous ethanol.

Pre-experiment Preparation and Important Notes

1. Add 5ml Proteinase K Storage Buffer to Proteinase K to dissolve it, and store it at –20°C. Do not leave the prepared Proteinase K at room temperature for a long time, and avoid repeated freezing and thawing to avoid affecting its activity.
2. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller DNA fragments and a decrease in the amount of extracted DNA.

3. This kit can extract up to 1–5 ml of whole blood samples, if you need to extract a large number of blood samples, please use the blood genome non-column extraction kit.

4. Anhydrous ethanol should be added to Buffer GW1 and Buffer GW2 according to the instructions on the label of the reagent bottle before first use.

5. Please check Buffer GL for crystallization or precipitation before use, if there is any crystallization or precipitation, please put it in 56°C water bath to re-dissolve.

6. If the downstream experiments are sensitive to RNA contamination, 4 µl of DNase Free RNase A (100mg/ml) can be added, RNase A is not provided in the kit, and can be ordered separately from our company if needed.

7. The Buffer RCL in the kit cannot be used further after turbidity.

procedure

1. Add 1–5 ml of blood sample to a centrifuge tube (supplied) and add 3 times the volume of Buffer RCL and gently vortex or invert to mix.

2. Centrifuge at 3000 rpm (~900 x g) for 10 minutes and carefully aspirate the supernatant.

3. Add 400 µl Buffer GR to the precipitate and resuspend the precipitate.

Note: If the downstream assay is sensitive to RNA, add 4 µl of RNase A (100 mg/ml) solution, shake for 15 seconds, and leave at room temperature for 5 minutes.

4. For 1–2 ml blood sample extraction, add 40 µl Proteinase K to the above solution and mix well; for 2–5 ml blood sample extraction, add 100 µl Proteinase K to the above solution and mix well.

5. Add 400 µl of Buffer GL, mix upside down 15 times, and vigorously vortex and shake for at least 1 minute.

Note: Do not add Proteinase K directly to Buffer GL.

6. Incubate at 70° C for 10 minutes, during which time mixing was inverted several times.

Note: 1) If the solution is not completely clear, add appropriate amount of Proteinase K and incubate. Extend the incubation time until the solution is completely clear.

2) The yield of DNA has been maximized by 10 minutes of incubation, and continued prolongation of the incubation time has no effect on DNA yield or purity.

7. Add 400 µl of anhydrous ethanol and mix upside down 10 times. Centrifuge briefly to concentrate the liquid on the walls and cap to the bottom of the tube.

8. Add all of the solution obtained in the previous step to the Spin Columns DL in the collection tube. If the solution cannot be added all at once, transfer it several times. centrifuge at 12,000 rpm (~13,400 x g) for 1 minute, pour off the waste liquid from the collection tube, and put the column back into the collection tube.

9. Add 500 μ l of Buffer GW1 to the adsorption column (check that anhydrous ethanol is added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note: It is recommended that step 9 be repeated if the sample being extracted is the blood genome of a species such as mice or monkeys from which hemoglobin is difficult to remove.

10. Add 500 μ l Buffer GW2 to the adsorption column (check that anhydrous ethanol is added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note: Step 10 can be repeated if further DNA purity is required.

11. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.)

12. Place the adsorption column in a new centrifuge tube, add 50–200 μ l of Buffer GE or sterilized water to the middle of the adsorption column overhanging the column, leave it at room temperature for 2–5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20°C .

Note: 1) If the downstream experiment is sensitive to pH or EDTA, you can use sterilized water for elution. The pH of the eluent has a great influence on the elution efficiency, if water is used as the eluent should ensure that its pH is 7.0–8.5 (you can use NaOH to adjust the pH of the water to this range), and the elution efficiency is not high when the pH is lower than 7.0.

2) Incubation at room temperature for 5 minutes prior to centrifugation increases yield.

3) Re-elution with an additional 50–200 μ l Buffer GE or sterilized water can increase the yield.

4) If the final concentration of DNA is to be increased, the DNA eluate obtained in step 12 can be re-spiked onto the adsorbent membrane and centrifuged at 12,000 rpm.

1min; if the elution volume is less than 200 μ l, the final concentration of DNA can be increased, but the total yield may be reduced. If the amount of DNA is less than 1 μ g, elution with 50 μ l Buffer GE or sterilized water is recommended.

5) Because DNA preserved in water is subject to acidic hydrolysis, for long-term storage, it is recommended that it be eluted with Buffer GE and stored at -20°C .