Blood Genome Column Small Volume Extraction Kit

Project number: B665530

Storage conditions: 2-8° C.

Product Content:

Component	B665530 50preps	B665530 200preps
BufferRCL	125mL	2×260mL
BufferGR	15mL	50mL
BufferGL	15mL	50mL
BufferGW1 (concentrate)	13mL	52mL
BufferGW2 (concentrate)	15mL	50mL
BufferGE	15mL	60mL
ProteinaseK	1.25mL	4×1.25mL
SpinColumnsDM withCollectionTubes	50	200

Product Introduction:

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, hemoconcentrated brown and yellow layers, lymphocytes, cell-free body fluids, etc. The product can process 0.1-1mL of whole blood, and the maximum yield can be up to 30μg, and the purified DNA can be obtained in sizes from 100bp to 50kb. It can purify DNA from 100bp to 50kb in size, with high yield and good quality of purified DNA, removing protein, pigment, lipid and other inhibitory impurity contamination to the maximum extent, and can be directly used in PCR, fluorescence quantitative PCR, enzyme digestion and SouthernBlot and other experiments.

Self-contained reagent: anhydrous ethanol.

Pre-experiment Preparation and Important Notes

1. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller DNA fragments and lower extraction volumes.

2. This kit can extract up to 0.1-1mL whole blood sample or 1×107 white blood cells.

3. Anhydrous ethanol should be added to BufferGW1 and BufferGW2 according to the instructions on the label of the reagent bottle before first use.

4. Check BufferGL for crystallization or precipitation before use; if crystallization or precipitation occurs, re-dissolve BufferGL by incubation at 56°

C in a water bath.

5. The BufferRCL in the kit cannot be used after turbidity.

Operation steps

1. Sample handling:

1a. When extracting a 200uL blood sample, add the sample to the centrifuge tube (provided) and proceed directly to the next step of the experiment.

1b. When the blood sample volume is less than 200 μL, add BufferGR to make up to 200 μL before proceeding to the next step of the experiment.

1c. When the volume of blood sample exceeds 200 μL, add 1 to 2.5 times the volume of BufferRCL and gently vortex or invert to mix.

Centrifuge at 12,000 rpm (~13,400 × g) for 1 min, carefully aspirate the supernatant, and repeat the above steps once if there is still red color in the precipitate. Then add 200 μL of BufferGR to the precipitate and shake until thoroughly mixed before proceeding to the next step of the experiment.

1d. if the blood sample being processed is the anticoagulated blood of an avian, bird, amphibian or lower organism with nucleated red blood cells

cells, blood sample volume of 5-20 μL can be added to BufferGR and replenished to 200 μl for subsequent experiments.

Note: If the downstream assay is sensitive to RNA, add 4 μL of RNaseA (100 mg/mL) solution, shake for 15 s, and leave at room temperature for 5 min. RNaseA is not supplied in the kit, but can be ordered separately from the company if needed, item no. CW0601S.

2. Add 20 μL of ProteinaseK to the above solution and mix well.

3. Add 200 μLBufferGL and shake until thoroughly mixed.

Note: Do not premix ProteinaseK and BufferGL.

4. Incubate at 56° C for 10 minutes, mixing several times with inversion.

Note: 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.

5. Add 200 μL of anhydrous ethanol and mix upside down several times. Centrifuge briefly to concentrate the liquid on the walls and cap to the bottom of the tube.

6. Add all of the solution obtained in step 5 to the SpinColumnsDM packed into the collection tube, if the solution cannot be added at one time, it can be transferred in several times. centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube and put the column back into the collection tube.

7. Add 500 μL of BufferGW1 to the column (check that anhydrous ethanol is added before use), centrifuge the column at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the column back into the collection tube.

Note: It is recommended that step 7 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.

8. Add 500 μL of BufferGW2 to the column (check that anhydrous ethanol is added before use) at 12,000 rpm.

Centrifuge for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note: Step 8 can be repeated if further DNA purity is required.

9. 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 the residual ethanol from the adsorbent column, which can interfere with subsequent enzymatic reactions (digestion, PCR). (etc.).

10. Place the adsorption column in a new centrifuge tube (supplied), add 50-200 μL of BufferGE 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℃.

Note: 1) If downstream experiments are sensitive to pH or EDTA, you can elute with sterilized water. The pH of the eluent has an effect on elution efficiency

If water is used as the eluent, its pH should be guaranteed at 7.0-8.5 (the pH of water can be adjusted to this range with NaOH), and the elution efficiency is not high when the pH is lower than 7.0.

2) If the final concentration of DNA is to be increased, the resulting DNA eluate can be re-spiked onto the adsorbent membrane, left at room temperature for 2-5 minutes, and centrifuged at 12,000 rpm for 1 minute.

(3) Because DNA preserved in water is affected by acidic hydrolysis, for long-term storage, it is recommended that it be eluted with BufferGE and stored at -20℃.