Blood Genome Non-Column Extraction Kit (0.1-20 ml)

Project number: F669942

Storage conditions: 2-8° C.

Products

individual parts making up a compound	200ml
Buffer FG1	2×260 ml
Buffer FG2	120ml
Buffer GE	60ml
Proteinase K	12.5mg
Proteinase K Storage Buffer	1.25ml

Products

This kit provides a rapid and flexible method for the extraction of total DNA, including genomic DNA and mitochondrial DNA, from fresh or frozen whole blood (samples treated with anticoagulants such as citrate, EDTA, or heparin). The product can flexibly process 0.1-20 ml of whole blood by using a non-centrifugal column method without the need for organic solvents such as phenol, chloroform, and other organic solvents, and effectively removes proteins, pigments, lipids, and other inhibitory impurities contamination. , lipids and other inhibitory impurity contamination. The whole process is operated in one tube, which reduces the risk of contamination and sample mix-up. The extracted DNA is of high yield and good quality, and can be directly used in PCR, fluorescence quantitative PCR, enzyme digestion and Southern Blot, and library construction experiments.

Product Features

-High purity: the extracted genomic DNA can be directly used in various experiments such as downstream PCR, fluorescent quantitative PCR, enzyme digestion and so on.
-Large extraction volume: DNA can be extracted from 0.1-20 ml of whole blood without the use of organic solvents such as phenol and chloroform.
-Strong compatibility: Suitable for processing a wide range of blood and cell samples.

Self-contained reagents: isopropyl alcohol, 70% ethanol.

Pre-experiment Preparation and Important Notes

1. Add the specified amount of Proteinase K Storage Buffer to Proteinase K to dissolve it and store it at -20℃. 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. All centrifugation operations are done at room temperature.

3. Repeated freezing and thawing of blood samples will result in smaller DNA fragments and a decrease in the amount of DNA extracted. Repeated freezing and thawing of the resulting genomic DNA should also be avoided as much as possible to avoid degradation. If genomic DNA from frozen blood is extracted, it is recommended to thaw it quickly in a 37° C water bath before subsequent operations.

4. Storage of blood samples:

1) Short-term storage: Blood samples with anticoagulant added can be stored at 2-8° C for up to 10 days. For some experiments, such as Southern hybridization, where complete full-length genomic DNA is required, please store the blood samples at 2-8° C for no more than 3 days, when the degradation of genomic DNA is less severe.

Long-term storage: Blood with anticoagulant should be stored at -70℃ (if high molecular weight DNA is extracted, we recommend using EDTA as anticoagulant).

procedure

I. Genome extraction from 100-900μl of whole blood (take 300μl of blood processing volume as an example)

1. Take 300μl of whole blood into a 2ml centrifuge tube (prepared by yourself), add 300μl (equal volume of the sample) of Buffer FG1, mix up and down for 5 times, centrifuge at 10,000×g for 30 seconds, discard the supernatant.

2. Add 450 μl (1.5 times the sample volume) of Buffer FG1 to the centrifuge tube and vortex to disperse the precipitate completely. centrifuge at 10,000 g for 30 seconds, discard the supernatant and place the tube upside down on a clean absorbent paper and leave for 2 minutes.

Note: In rare cases the precipitate may relax, so pour off the supernatant slowly. Inverting the centrifuge tube on blotting paper reduces the reflux of supernatant from the walls of the tube.

3. Prepare a mixture of Buffer FG2 and Proteinase K according to the attached table (100:1 ratio).

Note: It is best to prepare this mixture now and use it up within 1 hour after preparation.

4. Add 150 μl of Buffer FG2 with Proteinase K and vortex immediately until the solution is free of clumps. Note: 1) If more than one sample is being processed at the same time, vortex and shake immediately after adding the Buffer FG2/Proteinase K mixture, do not wait until all samples have been added.

(2) Usually, vortexing 3-4 times, each time for 5 seconds, can make the precipitate fully suspended, if you find that the precipitate contains colloidal material after vortexing, you can add 30 μl of Buffer FG2/Proteinase K mixture, and vortex again.

5. Incubate at 65° C for 10 minutes, mixing several times.

Note: If the color of the sample changes from red to olive green, the protein

digestion is complete.

6. Add 150 μl of isopropanol and mix thoroughly, upside down, until filamentous or clustered genomic DNA appears.

Note: It is very important to mix thoroughly with isopropanol to precipitate the DNA. If the sample has a low leukocyte content and the DNA may not be visible, turn the tube upside down at least 20 times to ensure complete precipitation.

7. Centrifuge at 10,000 x g for 5 minutes.

Note: If the precipitate is not firmly adherent to the wall, the centrifugation time can be extended or the centrifugation force can be increased as appropriate.

8. Discard the supernatant and place the tube upside down on clean blotting paper to drain.

Note: In some cases, the precipitate may not stick to the wall, so be careful not to aspirate the precipitate.

9. Add 300 μl of 70% ethanol, vortex for 5 seconds, centrifuge at 10,000 x g for 5 minutes and discard the supernatant.

Note: If the precipitate is not firmly attached to the wall, extend the centrifugation time or increase the centrifugation force appropriately.

10. Invert the centrifuge tube onto clean blotting paper for 5 minutes to ensure that the precipitate is in the tube.

Note: A white DNA precipitate is visible at the bottom of the tube and in rare cases the precipitate may be slack, so pour off the supernatant slowly.

11. Air dry the DNA precipitate until all liquid evaporates (at least 5 minutes).

Note: Ethanol residue can affect subsequent enzyme reactions (digestion, PCR, etc.), but avoid over-drying the DNA precipitate as over-drying will make the DNA difficult to dissolve.

12. Add 200 μl Buffer GE, vortex at low speed for 5 seconds, and incubate at 65° C for 1 hour to dissolve the DNA, flicking several times during the period to aid dissolution. Store the DNA at -20° C.

Note: If the DNA is not completely solubilized, it can be left at room temperature overnight.

II. Extraction of genome from 1-5 ml of whole blood (take 3 ml of blood processing volume as an example)

1. Take 3 ml of whole blood in a 15 ml centrifuge tube (self-provided), add 3 ml (equal volume of the sample) of Buffer FG1, mix up and down for 5 times, centrifuge at 2,500×g for 5 minutes, and discard the supernatant.

2. Add 4.5 ml (1.5 times the sample volume) of Buffer FG1 to the centrifuge tube, vortex and shake to disperse the precipitate completely. centrifuge for 5 minutes at 2,500 × g, discard the supernatant, and place the tube upside down on clean absorbent paper and leave it for 2 minutes.

Note: In rare cases the precipitate may relax, so pour off the supernatant slowly. Inverting the tube onto blotting paper will minimize reflux of supernatant from the tube wall.

3. Prepare a mixture of Buffer FG2 and Proteinase K (100:1) according to the attached table.

Note: It is best to use this mixture as it is, and to use it up within 1 h after preparation. 4. Add 1.5 ml of Buffer FG2 and Proteinase K to the mixture.

4. Add 1.5 ml of Buffer FG2/Proteinase K mixture and vortex immediately until the solution is free of clumps.

Note: 1) If more than one sample is being processed at the same time, vortex and shake immediately after adding Buffer FG2/Proteinase K mixture, do not wait until all samples have been added.

(2) Usually, vortexing 3-4 times, each time for 5 seconds, can make the precipitate fully suspended, if you find that the precipitate contains colloidal material after vortexing, you can add 300 μl of BufferFG2/Proteinase K mixture, and then vortex again to mix well.

5. Incubate at 65° C for 10-30 minutes, mixing several times.

Note: If the color of the sample changes from red to olive green it means the protein digestion is complete.

6. Add 1.5 ml of isopropanol and mix thoroughly by turning up and down until DNA is visible.

Note: It is important to mix thoroughly with isopropanol to precipitate the DNA. If the sample is low in leukocytes and the DNA may not be visible, turn the tube upside down at least 20 times to ensure complete precipitation.

7. Centrifuge at 2,500 x g for 5 minutes.

Note: If the precipitate is not firmly adherent to the wall, the centrifugation time can be extended or the centrifugation force increased as appropriate.

8. Discard the supernatant and place the tube upside down on clean blotting paper to drain.

Note: White DNA precipitate can be seen at the bottom of the tube. In rare cases, the precipitate may be loose, so pour off the supernatant slowly.

9. Add 1.5 ml of 70% ethanol, vortex and shake for 5 seconds, centrifuge at 2,500 x g for 5 minutes and discard the supernatant.

Note: If the precipitate is not firmly attached to the wall, you can extend the centrifugation time or increase the centrifugal force appropriately.

10. Invert the centrifuge tube onto clean blotting paper for 5 minutes to ensure that the precipitate is in the tube.

Note: In rare cases the precipitate may be slack, so pour off the supernatant slowly.

11. Air dry the DNA precipitate until all liquid has evaporated (at least 5 minutes).

Note: Ethanol residues can affect subsequent enzymatic reactions (digestion, PCR, etc.), but avoid over-drying the DNA precipitate, as over-drying can make the DNA difficult to dissolve.

12. Add 300μl of Buffer GE, vortex at low speed for 5 seconds, incubate at 65℃ for 1 hour to dissolve the DNA, and flick several times during the incubation to aid in dissolution. Store the DNA at -20° C.

Note: If the DNA is not completely solubilized, it can be left at room temperature overnight.

III. Extraction of genome from 6-20 ml of whole blood (take 10 ml of blood processing volume as an example)

1. Take 10 ml of whole blood in a 50 ml centrifuge tube (self-provided), add 10 ml of Buffer FG1, mix it up and down for 5 times, and centrifuge it at 2,500×g for 5 minutes.

2. Add 15 ml of Buffer FG1 to the centrifuge tube, vortex and shake to disperse the precipitate completely. centrifuge for 5 minutes at 2,500 x g. Discard the supernatant and place the tube upside down on clean blotting paper and leave for 2 minutes.

Note: In rare cases the precipitate may relax, so pour off the supernatant slowly.

3. Prepare a mixture of Buffer FG2 and Proteinase K according to the attached table (100:1 ratio).

Note: It is best to prepare this mixture now and use it up within 1h after preparation.

4. Add 5 ml of Buffer FG2/Proteinase K mixture and vortex immediately until the solution is free of clumps.

Note: 1) If more than one sample is handled at the same time, vortex and shake immediately after adding Buffer FG2/Proteinase K mixture, do not wait until all samples have been added.

(2) Usually, vortexing and shaking 3-4 times for 5 seconds each time can make the precipitate fully suspended. If the precipitate contains gelatinous material after vortexing and shaking, add 1ml of BufferFG2/Proteinase K mixture and vortex again to mix well.

5. Incubate at 65° C for 30 minutes, mixing several times.

Note: If the color of the sample changes from red to olive green, the protein digestion is complete.

6. Add 5 ml of isopropanol and mix thoroughly by turning up and down until DNA is visible.

Note: It is very important to mix thoroughly with isopropanol to precipitate the DNA, and the tube should be turned upside down at least 20 times to ensure complete precipitation.

7.2 Centrifuge at 500 x g for 5 minutes.

Note: If the precipitate is not firmly adherent to the wall, the centrifugation time can be extended or the centrifugation force can be increased.

8. Discard the supernatant and place the tube upside down on clean blotting paper to drain.

Note: White DNA precipitate can be seen at the bottom of the tube. In rare cases, the precipitate may be loose, so pour off the supernatant slowly.

9. Add 5 ml of 70% ethanol, vortex and shake for 5 seconds, centrifuge at 2,500 x g for 5 minutes and discard the supernatant.

Note that if the precipitate is not firmly adhered to the wall, you can extend the centrifugation time or increase the centrifugal force appropriately.

10. Place the centrifuge tube upside down on clean absorbent paper for 5 minutes to ensure that the precipitate is in the tube.

Note: In rare cases the precipitate may be slack, so pour off the supernatant slowly.

11. Air dry the DNA precipitate until all liquid has evaporated (at least 5 minutes).

Note: Ethanol residues can affect subsequent enzyme reactions (digestion, PCR,

etc.), but avoid over-drying the DNA precipitate as over-drying will make the DNA difficult to dissolve.

12. Add 1 ml of Buffer GE, vortex at low speed for 5 seconds, and incubate at 65° C for 1 hour to dissolve the DNA, flicking several times during the period to aid dissolution. Store the DNA at -20° C.

Note: If the DNA is not completely dissolved, it can be left at room temperature overnight.

Table: Amounts of various buffers required for different volumes of blood

	Volume of blood sample (μl)						
	100	300	1000	3000	5000	10000	20000
Buffer FG1 (μl)	250	750	2500	7500	12500	25000	50000
Buffer FG2 (μl)	50	150	500	1500	2500	5000	10000
Proteinase K (μl)	0.5	1.5	5	15	25	50	100
Isopropanol (μl)	50	150	500	1500	2500	5000	10000
70% ethanol (μl)	50	150	500	1500	2500	5000	10000
Buffer GE (μl)	100	200	200	300	500	1000	1000
Add a mixture of FG2 and Protein K	10	30	100	300	500	1000	1000