DNA extraction kit for blood film by magnetic bead method

Project number: M666110

Storage conditions: room temperature.

Product Content:

Component	M666110 96preps
BufferWSL	40mL
BufferMSL	40mL
BufferCW1 (concentrate)	90mL
BufferGW1 (concentrate)	40mL
BufferGW2 (concentrate)	50mL
BufferEB	30mL
ProteinaseK	4×1.25mL
MagbeadsV3	2×1mL

Product Introduction

The kit provides a simple, rapid, and efficient method for extracting DNA from blood films and is suitable for extracting genomic DNA from blood films. In the presence of high salt, DNA is bound to the surface of silica-coated Magbeads. After rinsing, the highly pure DNA is eluted in BufferEB or deionized water. The DNA obtained from purification has good purity (A260/280 ratio between 1.7-1.9) and high integrity (>15kb), and can be used for downstream experiments such as second-generation sequencing, quantitative PCR, and microarray detection.

Bring your own instruments and reagents

1) Thermostatic homogenizer
2) 2/15ml magnetic rack
3) 32-channel nucleic acid extractor
4) 96-channel nucleic acid extractor
5) 96DWPlate
6) 8channelComb
7) Spintipspack
8) Anhydrous ethanol

Pre-experiment Preparation and Important Notes

1. Add anhydrous ethanol to BufferCW1, BufferGW1 and BufferGW2 according to the label of the reagent bottle and label it before the first use.

2. Freezing and centrifugation of Magbeads is strictly prohibited. Freezing and centrifugation may cause irreversible damage to Magbeads.

procedure

Ⅰ, manual single-tube operation

1. Take one 6 mm diameter blood spot or four 3 mm diameter blood spots (as appropriate) from the blood spot into a 2.0 mL centrifuge tube using punching forceps.

2. Add 40 μL of ProteinaseK and 300 μL of BufferWSL to the centrifuge tube, after which the tube was placed on a thermostatic mixer at 75° C and 1200 rpm for 45 minutes to form Lysate by shaking and lysing, the tube was removed from the thermostatic mixer, briefly centrifuged, and the supernatant was removed.

Note: If a thermostatic mixer is not available, incubate the centrifuge tubes in a 75° C water bath for 30 minutes after vortexing and shaking for 10 seconds at 10-minute intervals.

Minute vortex shaking for 10 seconds.

3. The supernatant was pipetted into a new 2.0 mL centrifuge tube and 300 μL of BufferMSL, 300 μL of isopropanol and 20 μL of MagbeadsV3 were added, after which the tube was shaken and lysed for 15 minutes at 25° C on a thermostatic mixer at 1,600 rpm, or the tube was mixed continuously in an inverted position for 15 minutes.

4. Place the centrifuge tube on the magnetic rack for 1 minute and discard the solution well after the Magbeads are completely adsorbed on the side walls of the tube (keep the tube fixed on the magnetic rack).

5. Remove the centrifuge tube from the magnetic rack, add 900 μL of BufferCW1 (check that anhydrous ethanol has been added before use) and vortex for 1 minute or vortex for 5 seconds, then place the tube on a thermostatic mixer at 25℃ and 1600 rpm for 2 minutes (make sure that the Magbeads are in the mixing state during the process of vortexing). Afterwards, the centrifuge tube was placed on a magnetic rack for 1 minute, and after the Magbeads were completely adsorbed to the sidewalls of the centrifuge tube, the magnetic rack was gently inverted to wash the impurities from the cap of the centrifuge tube, and the solution was discarded completely (the centrifuge tube was kept fixed on the magnetic rack).

6. Remove the centrifuge tube from the magnetic rack, add 500 μL of BufferGW1 (check that anhydrous ethanol has been added before use) and vortex for 1 minute or vortex for 5 seconds, then place the tube on a thermostatic mixer at 25℃ and 1600 rpm for 2 minutes (make sure that the Magbeads are in the mixing state during the process of vortexing). Afterwards, the centrifuge tube was placed on a magnetic rack for 1 minute, and after the Magbeads were completely adsorbed to the sidewalls of the centrifuge tube, the magnetic rack was gently inverted to wash the impurities from the cap of the centrifuge tube, and the solution was discarded completely (the centrifuge tube was kept fixed on the magnetic rack).

7. Remove the centrifuge tube from the magnetic rack, add 900 μL of BufferGW2 (check that anhydrous ethanol has been added before use) and vortex for 1 minute or vortex for 5 seconds, then place the tube on a thermostatic mixer at 25℃ and 1600 rpm for 2 minutes (make sure that the Magbeads are in the mixing state during the process of vortexing). Afterwards, the centrifuge tube was placed on a

magnetic rack for 1 minute, and after the Magbeads were completely adsorbed to the side wall of the centrifuge tube, the magnetic rack was gently inverted to wash the impurities from the cap of the centrifuge tube, and the solution was completely discarded (the centrifuge tube was kept fixed on the magnetic rack).

8. Remove the centrifuge tube from the magnetic rack, add 300 μL of 75% ethanol and vortex for 1 minute or vortex for 5 seconds, and then place the tube on a thermostatic mixer at 25℃ and 1600 rpm for 2 minutes (make sure that the Magbeads are in the mixing state during the process of vortexing). Afterwards, the centrifuge tube was placed on a magnetic rack for 1 minute, and after the Magbeads were completely adsorbed to the sidewalls of the centrifuge tube, the magnetic rack was gently inverted to wash the impurities from the cap of the centrifuge tube, and the solution was completely discarded (the centrifuge tube was kept fixed on the magnetic rack).

9. Keeping the centrifuge tubes fixed on a magnetic rack, use a pipette to further remove the solution from the bottom and cap of the centrifuge tubes, after which they are left at room temperature for 5-10 minutes to allow the ethanol to evaporate.

10. Remove the centrifuge tube from the magnetic rack and add 50-200 μL of BufferEB. Vortex the beads to completely suspend them in the eluent and then elute them by shaking them for 10 minutes on a thermostatic mixer at 56° C and 1600 rpm or incubate the tube for 10 minutes in a water bath at 56° C, vortexing the tube for 10 seconds at 3-minute intervals during the incubation period.

11. Place the centrifuge tube on a magnetic rack and let it stand for 2 minutes. After the Magbeads are completely adsorbed on the side wall of the centrifuge tube, use a pipette to transfer the eluate to a new centrifuge tube and store it at -20℃.

II. Matching with CWE2100

1. Take one 6 mm diameter blood spot or four 3 mm diameter blood spots (as appropriate) from the blood spot into a 2.0 mL centrifuge tube using punch pliers.

2. 40 μL of ProteinaseK and 300 μL of BufferWSL were added to the centrifuge tube, after which the tube was shaken and lysed on a thermostatic mixer at 75° C and 1200 rpm for 45 min to form Lysate.

3. Add the appropriate reagents to the 96DW deep well plate according to the table below.

Position	Reagent
1&7Colume	Lysate:All BufferMSL:300 μL Isopropyl alcohol:300 μL MagbeadsV3:20 μL
2&8Colume	BufferCW1:900 μL
3&9Colume	BufferGW1:500 μL
4&10Colume	BufferGW2:900 μL
5&11Colume	75% ethanol: 300 μL
6&12Colume	BufferEB:70 μL

4. Put the reagent-added deep-well plate and magnetic sleeve in the corresponding position of CWE2100/CWE3200, run the blood film extraction program, and take out the deep-well plate and magnetic sleeve after about 40 minutes of the end of the program.

5. Transfer the elution products in columns 6&12 of the deep-well plate to a 1.5mL centrifuge tube for cryopreservation.

III, matching CWE960

1. Take one 6 mm diameter blood spot or four 3 mm diameter blood spots (as appropriate) from the blood spot into a 2.0 mL centrifuge tube using punch pliers.

2. 40 μL of ProteinaseK and 300 μL of BufferWSL were added to the centrifuge tube, after which the tube was shaken and lysed on a thermostatic mixer at 75° C and 1200 rpm for 45 min to form Lysate.

3. Add the appropriate reagents to the 96DW deep well plate according to the table below.

Position	Reagent
Plate1	Lysate:All BufferMSL:300μL Isopropyl alcohol:300μL MagbeadsV3:20μL
Plate2	BufferCW1:900μL
Plate3	BufferGW1:500μL
Plate4	BufferGW2:900μL
Plate5	75% ethanol: 300μL
Plate6	BufferEB:70μL

4. Put the reagent-added deep-well plate and magnetic sleeve in the corresponding position of the CWE960, run the blood film extraction program, and take out the deep-well plate and magnetic sleeve after about 40 minutes of the end of the program.

5. Transfer the elution product in Plate6 to a 1.5 mL centrifuge tube for cryopreservation.