Version: 12/2023

Magbead Blood Spots DNA Kit

Cat. No. : CW2504S (96 preps)

Storage Conditions: Room temperature (15-30°C)

Components

Component	CW2504S 96 preps
Buffer WSL	40 mL
Buffer MSL	40 mL
Buffer CW1 (concentrate)	90 mL
Buffer GW1 (concentrate)	40 mL
Buffer GW2 (concentrate)	50 mL
Buffer EB	30 mL
Proteinase K	4×25 mg
Proteinase K Storage Buffer	4×1.25 mL
Magbeads SN	2×1 mL

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

Introduction

The kit provides a simple, rapid and efficient method for the extraction of genomic DNA from blood spots. In the presence of high salt, DNA is bound to the surface of silica-coated Magbeads. After rinsing, the high purity DNA is eluted in Buffer EB or deionised water. The purified DNA is of good purity (A260/280 ratio between 1.7 and 1.9) and high integrity (>15 kb) and can be used for downstream experiments such as second-generation sequencing, quantitative PCR and chip detection.

Reagents to Be Supplied by User

1. Thermostatic Mixer
2. Magnetic stand
3. 32-Channel Nucleic Acid Extractor
4. 96-Channel Nucleic Acid Extractor
5. 96 DW Plate
6. 8 channel Comb
7. Spin tips pack
8. 100% ethanol

Precautions

1. Add 1.25 mL Proteinase K Storage Buffer to 25 mg Proteinase K to dissolve it and store at -20°C. Do not store the prepared Proteinase K at room temperature for a long time, and avoid repeated freezing and thawing, so as not to affect its activity.
2. Before first use add 100% ethanol to Buffer CW1, Buffer GW1 and Buffer GW2 according to the labels of the reagent bottles and mark them well.
3. Magbeads should not be frozen or centrifuged at high speed as this may cause irreversible damage to Magbeads.

iii Matched to CWE960

1. Take one blood spot with a diameter of 6 mm or four blood spots with a diameter of 3 mm (according to the actual situation) from the blood spot with a punching pliers and put them into a 2.0 mL centrifuge tube.
2. Add 40 μL Proteinase K and 300 μL Buffer WSL to the centrifuge tube, then put the centrifuge tube on a thermostatic mixer at 75℃ and 1200 rpm for 45 minutes to form Lysate.
3. Add the appropriate reagents to the 96 DW Plate according to the table below.

Position	Reagent
Plate 1	Lysate: All Buffer MSL: 300 μL Isopropanol: 300 μL Magbeads SN: 20 μL
Plate 2	Buffer CW1: 900 μL
Plate 3	Buffer GW1: 500 μL
Plate 4	Buffer GW2: 900 μL
Plate 5	75% Ethanol: 300 μL
Plate 6	Buffer EB: 70 μL

4. Place the plate and comb in the corresponding position on the CWE960, run the blood spots extraction program. After approximately 40 minutes, take out the plate and comb.
5. Transfer the eluted product from Plate 6 to 1.5 mL centrifuge tubes and store at low temperature.

Protocol

i Manual single pipe operation

1. Take one blood spot with a diameter of 6 mm or four blood spots with a diameter of 3 mm (according to the actual situation) from the blood spot with a punching pliers and put them into a 2.0 mL centrifuge tube.
2. Add 40 μL Proteinase K and 300 μL Buffer WSL to the centrifuge tube, then put the centrifuge tube on a thermostatic mixer at 75°C and 1200 rpm for 45 minutes to form Lysate, take the centrifuge tube off the thermostatic mixer, centrifuge briefly, and take the supernatant.

   Note: If there is no constant temperature mixer, vortex shake the tube for 10 seconds and incubate in a 75°C water bath for 30 minutes, vortex shaking for 10 seconds at 10 minute intervals.
3. Transfer the supernatant to a new 2.0 mL centrifuge tube and add 300 μL Buffer MSL, 300 μL of isopropanol, and 20 μL of Magbeads SN. The tube is then shaken and lysed at 25°C for 15 minutes on a thermostatic mixer at 1600 rpm or the tube is continuously inverted and mixed for 15 minutes.
4. Place the centrifuge tube on the magnetic stand for 1 minute and allow the Magbeads to fully attach to the side walls of the tube and then fully discard the solution (keeping the tube fixed to the magnetic stand).
5. Take out the centrifuge tube from the magnetic stand, add 900 μL Buffer CW1 (please check whether 100% ethanol is added before use) and vortex shake for 5 seconds, and then put on a thermostatic mixer at 25°C and 1600 rpm for 2 minutes (ensure that the Magbeads are mixed during the shaking process). Then, the centrifuge tube is placed on the magnetic stand for 1 minute. After Magbeads is completely adsorbed on the side wall of the centrifuge tube, the magnetic stand is gently inverted to wash the impurities on the centrifuge tube cover and completely discard the solution (the centrifuge tube is kept fixed on the magnetic stand).

6. Take out the centrifuge tube from the magnetic stand, add 500 μL Buffer GW1 (please check 100% anhydrous ethanol is added before use) and vortex shake for 5 seconds, and then put on a thermostatic mixer at 25°C and 1600 rpm for 2 minutes (ensure that the Magbeads are mixed during the shaking process). Then, the centrifuge tube is placed on the magnetic stand for 1 minute. After Magbeads was completely adsorbed on the side wall of the centrifuge tube, the magnetic stand is gently inverted to wash the impurities on the centrifuge tube cover and completely discard the solution (the centrifuge tube was kept fixed on the magnetic stand).
7. Take out the centrifuge tube from the magnetic stand, add 900 μL Buffer GW2 (please check whether 100% ethanol is added before use) and vortex shake for 5 seconds, and then put on a thermostatic mixer at 25°C and 1600 rpm for 2 minutes (ensure that the Magbeads are mixed during the shaking process). Then, the centrifuge tube is placed on the magnetic stand for 1 minute. After Magbeads was completely adsorbed on the side wall of the centrifuge tube, the magnetic stand is gently inverted to wash the impurities on the centrifuge tube cover and completely discard the solution (the centrifuge tube was kept fixed on the magnetic stand).
8. Take out the centrifuge tube from the magnetic stand, add 300 μL 75% ethanol and vortex shake for 5 seconds, and then put on a thermostatic mixer at 25°C and 1600 rpm for 2 minutes (ensure that the Magbeads are mixed during the shaking process). Then, the centrifuge tube is placed on the magnetic stand for 1 minute. After Magbeads was completely adsorbed on the side wall of the centrifuge tube, the magnetic stand is gently inverted to wash the impurities on the centrifuge tube cover and completely discard the solution (the centrifuge tube was kept fixed on the magnetic stand).
9. Keep the tube on a magnetic stand and use a pipette to further remove the solution from the bottom and cap of the tube, then leave at room temperature for 5-10 minutes to allow the ethanol to evaporate.

10. Take out the tube from the magnetic stand and add 50-200 μL of Buffer EB. Vortex shake to completely suspend the magbeads in the eluate and put on a thermostatic mixer at 56°C and 1600 rpm for 10 minutes or incubate the tube in a 56°C water bath for 10 minutes, vortex shaking for 10 seconds at 3 minutes intervals.
11. Place the centrifuge tube on a magnetic stand for 2 minutes, then transfer the eluate to a new centrifuge tube and store at -20°C.

ii Matched to CWE2100

1. Take one blood spot with a diameter of 6 mm or four blood spots with a diameter of 3 mm (according to the actual situation) from the blood spot with a punching pliers and put them into a 2.0 mL centrifuge tube.
2. Add 40 μL Proteinase K and 300 μL Buffer WSL to the centrifuge tube, then put the centrifuge tube on a thermostatic mixer at 75℃ and 1200 rpm for 45 minutes to form Lysate.
3. Add the appropriate reagents to the 96 DW Plate according to the table below.

Position	Reagent
Colume 1&7	Lysate: All Buffer MSL: 300 μL Isopropanol: 300 μL Magbeads SN: 20 μL
Colume 2&8	Buffer CW1: 900 μL
Colume 3&9	Buffer GW1: 500 μL
Colume 4&10	Buffer GW2: 900 μL
Colume 5&11	75% Ethanol: 300 μL
Colume 6&12	Buffer EB: 70 μL

4 Place the plate and comb in the corresponding position on the CWE2100/CWE3200, run the blood spots extraction program. After approximately 40 minutes, take out the plate and comb.
5. Transfer the eluted product from columns 6 & 12 of the deep well plate to 1.5 mL centrifuge tubes and store at low temperature.