13. Add 30-100 μL RNase-free Water to the centrifugal tube, remove the centrifugal tube from the magnetic rack, vortex it and make the magbeads completely suspended in the eluent. And then put them on the Thermomixer of 56°C and 1600 rpm for 10 min.

    Note: 1) In general, increasing the elution volume can increase the DNA yield, decreasing the elution volume can increase the DNA concentration. It is suggested that the elution volume should be determined according to the needs of subsequent experiments.

    2)If the magbeads are not vortexed to a fully dispersed state, the extraction rate will be reduced.

    3)If a Thermomixer is not available, incubate the centrifuge tube at 65°C in a water or metal bath for 15 min, vortexing for 5 s every 2 min.

14. Place the centrifuge tube on a magnetic rack for 2 minutes, then transfer the eluate to a new centrifuge tube for cryopreservation.

Compatible with CWE960

1. Add samples and reagents to the corresponding positions according to the following table:

Name	Type	Regents & Volume
Sample Plate	DW 96 deep-well plate	Proteinase K:13 μL Plasma: 200 μL Buffer KPL: 320 μL Magbeads:17 μL 96 DW Comb
Wash Plate I	DW 96 deep-well plate	Buffer GW1: 500 μL
Wash Plate II	DW 96 deep-well plate	Buffer GCW2: 500 μL
Wash Plate III	DW 96 deep-well plate	Buffer GCW2: 500 μL
Elution Plate	DW 96 deep-well plate	RNase-free Water : 70 μL

Note: please add the reagents in order.

2. Turn on the CWE960, run the CW2522 program, and follow the instrument's instructions to place the deep-well plate and the magnetic sleeve into the instrument.
3. Remove the deep-well plate when the program has finished running. Close the Elution Plate with a sealing film and store it frozen.

Version: 10/2023

Magbead Free-Circulating DNA Kit

Cat. No. : CW2522S (96 preps)

Storage Condition: Magbeads ZN is stored at 2-8°C and the other components are stored at room temperature.

Components

Component	CW2552S (96 preps)
Buffer KPL	55 mL
Buffer GW1（concentrate）	30 mL
Buffer GCW2（concentrate）	20 mL
RNase-free Water	30 mL
Proteinase K	2×1.25 mL
Magbeads ZN	3×1 mL

Introduction

This kit is suitable for the purification and recovery of cell-free DNA (Free-circulating/ Cell-free DNA) from cell-free body fluids such as plasma, serum and amniotic fluid. At high salt, cell-free DNA binds to the surface of silica-coated magbeads. After rinsing, the cell-free DNA is eluted in RNase-free Water. The yield of cell-free DNA is closely related to sample type, storage conditions, time and individual difference. The quality of cell-free DNA obtained by purification is stable and reliable, and can be used for qPCR, prenatal diagnosis and other downstream experiments.

The kit can be used with magnetic rod method magbead automatic purification system and liquid workstation to carry out high-throughput extraction simply and quickly, which greatly reduces the workload of the experimenter and the human error in the experiment.

Equipment and Reagents to be Supplied by user

1. CWE960, or other automatic nucleic acid extractor
2. Thermomixer
3. 100% ethanol

Precautions

1. Samples should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be small and the extraction yield will be low.
2. Before first use, add 100% ethanol to Buffer GW1 and Buffer GCW2 and mark them.
3. Freezing and high-speed centrifugation of Magbeads ZN are strictly prohibited, otherwise it may cause irreversible damage to Magbeads ZN.
4. Magbeads ZN should be vortexed for 30 s to mix well before use.

Protocol

Manual

1. Add 13 μL of Proteinase K to a 1.5 mL centrifuge tube.
   Add 200 μL of serum/plasma sample to the centrifuge tube from the previous step.
   Note: The frozen samples need to be lysed in advance by placing them in a 4°C refrigerator. Repeatedly invert the sample storage tubes during the lysis process to mix the samples.
2. Add 320 μL of lysis Buffer KPL to the centrifuge tube from the previous step and vortex for 5 s to mix well.
3. Place the centrifuge tube on a Thermomixer at 56°C, 1300 rpm and vortex the lysis for 10 min.
   Note: 1) If Thermomixer is not available, incubate the centrifuge tube in a water or metal bath at 56°C for 10 min, during which time it should be vortexed and shaken for 5 s every 2 min.
   2) If the sample volume is greater or less than 200 μL, the volume of Buffer KPL and magbead suspension should be adjusted proportionally, and the volume of magbead suspension should not be less than 10 μL.
4. Add 17 μL of magbead suspension to the centrifuge tube in the previous step (the magbead suspension should be vortexed for 10 s before adding to make it well mixed), vortex for 5 s to make it mixed well, and then put the centrifuge tube on a Thermomixer at 25°C and 1300 rpm for 5 min.
   Note: If a Thermomixer is not available, mix the centrifuge tube continuously upside down for 10 min.

5. Place the centrifuge tube from the previous step on the magnetic rack and rest for 1 min, discard the solution completely when the magbeads are fully adsorbed on the side wall of the tube (keep the tube fixed on the magnetic rack), and avoid touching the magbeads.
6. After adding 500 μL Buffer GW1 to the centrifugal tube (check whether 100% ethanol is added before adding), remove the centrifugal tube from the magnetic rack, vortex it for 5 s, and then place the tube on a Thermomixer at 25°C, 1600 rpm for 2 min.
   Note: If a Thermomixer is not available, the tube can be vortexed for 10 s to fully suspend the magbeads in the in the Buffer GW1.
7. Place the centrifuge tube from the previous step on the magnetic rack for 1 min. After the magbeads are completely absorbed on the side wall of the centrifugal tube, gently invert the magnetic rack to wash the impurities from the centrifuge tube cap and discard the solution thoroughly (keep the tube fixed on the magnetic rack), avoiding contact with the magbeads.
   Note: If any residual solution on the cap of the centrifuge tube when the solution is discarded, use a pipette to further remove it.
8. Add 500 μL of Buffer GCW2 to the centrifuge tube (check whether 100% ethanol is added before adding), remove the tube from the magnetic rack, vortex it for 5 s, and then place the tube on a Thermomixer at 25°C, 1600 rpm for 2 min.
   Note: If a Thermomixer is not available, the tube can be vortexed for 10 s to fully suspend the magbeads in the in the Buffer GCW2.
9. Place the centrifuge tube from the previous step on the magnetic rack for 1 min. After the magbeads are completely absorbed on the side wall of the centrifugal tube, gently invert the magnetic rack to wash the impurities from the centrifuge tube cap and discard the solution thoroughly (keep the tube fixed on the magnetic rack), avoiding contact with the magbeads.
   Note: If any residual solution on the cap of the centrifuge tube when the solution is discarded, use a pipette to further remove it.
10. Repeat step 8 and step 9.
11. Remove the centrifuge tube from the magnetic rack and centrifuge with a short time, then put the centrifuge tube back on the magnetic rack, and use the pipette to further remove the solution at the bottom of the tube.
12. Keep the centrifuge tube fixed on a magnetic rack for 5-10 min at room temperature to allow the ethanol to evaporate cleanly.