Compatible with CWE240(Take 4 mL plasma as an example)

1. Add samples and reagents to the 24 DW Plate according to the following table:

Name	Regents & Volume (2 mL)	Regents & Volume (4 mL)
Plate 1	Proteinase K: 30 μL Plasma: 2 mL 20% SDS: 100 μL	Proteinase K: 60 μL Plasma: 4 mL 20% SDS: 200 μL
Plate 2	Buffer GW1: 1 mL	Buffer GW1: 1 mL
Plate 3	Buffer GW1: 1 mL	Buffer GW1: 1 mL
Plate 4	Buffer GCW2: 1 mL	Buffer GCW2: 1 mL
Plate 5	Buffer GcW2: 1 mL	Buffer GcW2: 1 mL
Plate 6	RNase-Free Water: 100 μL	RNase-Free Water: 100 μL

2. Put the "24 DW Plate and Tips Pack for CWE240" into the corresponding positions of the CWE240 nucleic acid extractor, and run the "CW2560 ctDNA program".
3. After about 25 minutes, the instrument is paused, and the "Plate 1" is taken out of the instrument and placed on ice for 5-10 minutes, then press the table below to add the reagent.

Name	Regents & Volume (2 mL)	Regents & Volume (4 mL)
Plate 1	Buffer MPL: 2 mL isopropanol: 0.5 mL Magbeads ZN: 30 μL	Buffer MPL: 4 mL isopropanol: 1 mL Magbeads ZN: 60 μL

4. Put the 24 DW plate back into the instrument and continue running the program. The program ends after about 45 minutes. Transfer the DNA elution product from "Plate 6" to a centrifuge tube and store at -20°C for later use.

Version: 12/2023

Magbead Free-Circulating DNA Maxi Kit

Cat. No. : CW2560S (2 mL×48 preps)

Storage Condition: Magbeads ZN at 2-8°C and other components at room temperature (15-30°C).

Components

Component	CW2560S 2 mL×48 preps
Buffer MPL	120 mL
20% SDS	6 mL
Buffer GW1（concentrate）	80 mL
Buffer GCW2（concentrate）	40 mL
RNase-Free Water	30 mL
Proteinase K	2×1.25 mL
Magbeads ZN	2×1 mL

Principle

This kit is suitable for purifying cell-free DNA (Free-circulating/cell-free DNA) from plasma, serum, amniotic fluid and other cell-free body fluids. Cell-Free DNA binds to the surface of silica-coated magbeads in a high-salt state, and is eluted in RNase-Free Water after rinsing. The yield of cell-free DNA is dependent on sample type, storage conditions, time, and inter-individual variability. The purified cell-free DNA has stable and reliable quality and can be used for quantitative PCR, prenatal diagnosis, etc.

Reagents and equipments to Be Supplied by User

100% ethanol
Isopropanol
CWE240 and other automatic nucleic acid extractor
24 DW Plate and Tips Pack for automatic nucleic acid extractor

Precautions

1. 100% ethanol should be added to the Buffer GW1 according to the label of the reagent bottle before the first use.
2. 100% ethanol should be added to the Buffer GCW2 according to the label of the reagent bottle before the first use.
3. If operated manually, preheat the constant temperature mixer to 60°C before the start of the experiment.
4. Freezing and high-speed centrifugation are strictly prohibited for Magbeads ZN, otherwise it may cause irreversible damage to Magbeads ZN. Magbeads ZN should fully oscillate and mix evenly each time you use it.
5. Please check Buffer MPL and 20% SDS for crystallization or precipitation before use. If there is crystallization or precipitation, you can resume clarification after bathing in 37°C for a few minutes.

Protocol (Manually, take 2 mL plasma as an example)

1. Add 30 μL Proteinase K, 2 mL plasma, 100 μL 20% SDS to the centrifuge tube in sequence according to the table below. And then put it at 60°C, 1200 rpm constant temperature mixer and shake for 20 minutes, after the incubation, put the centrifuge tube in an ice bath for 5-10 minutes.
   Note: if there is no constant temperature mixer, the centrifugal tube vortex is oscillated for 10 seconds and then incubated in a 60°C water bath for 20 minutes, during which the vortex oscillates for 10 seconds every 7 minutes.
   To avoid proteinase K inactivation, please add the reagents in the order shown in the table below. Do not add SDS directly to the proteinase K solution.

Regent	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
Proteinase K	15 μL	30 μL	60 μL	150 μL
Plasma	1 mL	2 mL	4 mL	10 mL
20% SDS	50 μL	100 μL	200 μL	500 μL
Total volume	1.065 mL	2.13 mL	4.26 mL	10.65 mL

2. During the incubation, prepare the lysate/magnetic bead mixture according to the table below and mix well.

Regent	Plasma volume			
	1 mL	2 mL	4 mL	10 mL
Buffer MPL	1 mL	2 mL	4 mL	10 ml
isopropanol	0.25 mL	0.5 mL	1 mL	2.5 mL
Magbeads ZN	15 μL	30 μL	60 μL	150 μL
Total volume	1.265 mL	2.53 mL	5.06 mL	12.65 mL

3. Add the lysate/magnetic bead mixture prepared in step 2 to the sample tube in step 1. Vortex for 1 minute, and mix upside down for 5-10 minutes to keep the magnetic beads in suspension.
4. Put the centrifuge tube on the magnetic stand and let it stand. After the magnetic beads are adsorbed on the magnetic stand, and the solution in the tube becomes clear, turn the centrifuge tube over to rinse the remaining magnetic beads on the cap of the bottle, and leave it for about 1 minute, then discard the solution.
5. Add 1 mL of Buffer GW1 to the centrifuge tube (please check whether 100% ethanol has been added before use), shake and mix, and then transfer the suspension to a new 1.5 mL centrifuge tube.
6. Place the centrifuge tube on the magnetic stand for 1 minute, then discard the solution.
7. Add 1 mL of Buffer GW1 to the centrifuge tube (please check whether 100% ethanol has been added before use), vortex for 5 seconds, and place it on a thermomixer at 25°C and 1600 rpm for 2 minutes.
8. Place the centrifuge tube on the magnetic stand for 1 minute, then discard the solution.
9. Add 1 mL of Buffer GCW2 to the centrifuge tube (please check whether 100% ethanol has been added before use), vortex for 5 seconds, then place it on a constant temperature mixer at 25°C and 1600 rpm for 2 minutes.
10. Place the centrifuge tube on the magnetic stand for 1 minute, then discard the solution.
11. Repeat steps 9-10.
12. After a brief centrifugation of the centrifuge tube, re-fix it on the magnetic stand, remove the solution at the bottom of the tube with a pipette, open the lid and place at room temperature for 5-10 minutes to fully evaporate the ethanol.
13. Add 50-100 μL RNase-Free Water to the centrifuge tube, vortex and shake to fully suspend the magnetic beads in the eluent, then fix the centrifuge tube on a constant temperature mixer at 25°C and 1600 rpm, shake and elute for 10 minutes.
14. Fix the centrifuge tube on the magnetic stand and let it stand for 2 minutes. After the Magbeads are completely adsorbed on the side wall of the centrifuge tube, transfer the eluate to a new centrifuge tube with a pipette and store at -20°C for later use.