Large Volume Free DNA Extraction Kit by Magnetic Bead Method

Project number: M670148

Storage conditions: room temperature.

Product Content:

Component	M670148 2 mL x 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 x 1.25 mL
Magbeads ZN	2 x 1 mL

Product Introduction

This kit is suitable for the purification and recovery of free-circulating/Cell-free DNA from cell-free fluids such as plasma, serum and amniotic fluid. At high salt, free DNA is bound to the surface of silica-coated magnetic beads. After rinsing, free DNA is eluted in RNase-Free Water. The yield of free DNA is highly dependent on the type of sample, storage conditions, time and inter-individual variability. The purified free DNA is of stable and reliable quality and can be used in downstream experiments such as quantitative PCR and prenatal diagnosis.

Bring your own instruments and reagents

1. Fully automated nucleic acid extractor
2. Anhydrous ethanol, isopropanol
3. 24 DW Plate and Tips Pack

Pre-experiment Preparation and Important Notes

1. Anhydrous ethanol should be added to Buffer GW1 before the first use according to the reagent bottle label instructions.
2. Anhydrous ethanol should be added to Buffer GCW2 before the first use according to the reagent bottle label instructions.
3. If operating manually, preheat the thermostatic mixer to 60° C before starting the experiment.
4. Magbeads ZN should not be frozen or centrifuged at high speeds as this may cause irreversible damage to Magbeads ZN. Magbeads ZN should be shaken well and mixed well each time it is used.
5. Check Buffer MPL and 20% SDS for crystallization or precipitation prior to use. If crystallization or precipitation is present, a water bath at 37° C for a few minutes will restore clarity.

Procedure (manual, using 2mL of plasma as an example)

1. Add 30 μL of Proteinase K, 2 mL of plasma, and 100 μL of 20% SDS to the centrifuge tube according to the table below, and place the tube on a thermostatic mixer at 60° C and 1200 rpm for 20 min. After incubation, place the tube in an ice bath for 5-10 min.
Note: If a thermostatic mixer is not available, vortex the centrifuge tube for 10 seconds and incubate in a 60° C water bath for 20 minutes at 7-minute intervals.
The clock vortex oscillates for 10 seconds.
To avoid inactivation of proteinase K, 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	10mL
Proteinase K	15 μL	30 μL	60 μL	150 μL
Plasma samples	1 mL	2 mL	4 mL	10mL
20% SDS	50 μL	100 μL	200 μL	500 μL
total volume	1.065mL	2.13 mL	4.26mL	10.65mL

2. During incubation, prepare the lysate/magnetic bead mix 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 mix prepared in Step 2 to the Step 1 sample tube. Vortex and shake for 1 minute, then mix by hand up and down or using a mixer for 5-10 minutes to keep the magnetic beads in suspension at all times.
4. Place the centrifuge tube on a magnetic rack and allow the beads to attach to the rack and the solution in the tube to become clear, then turn the tube over to rinse the beads from the cap and allow to stand for about 1 minute, after which the solution is discarded.
5. Add 1 mL of Buffer GW1 to the centrifuge tube (check that anhydrous ethanol has been added before use), shake to homogenize and transfer the suspension to a new 1.5 mL centrifuge tube.
6. Centrifuge tubes were fixed on a magnetic rack and left to stand for 1 minute, after which the solution was discarded.
7. Add 1 mL of Buffer GW1 to the centrifuge tube (check that anhydrous ethanol

has been added prior to use), vortex and shake for 5 seconds, then place on a thermostatic mixer at 25° C and 1600 rpm for 2 minutes.
8. Centrifuge tubes were fixed on a magnetic rack and left to stand for 1 minute, after which the solution was discarded.
9. Add 1 mL of Buffer GCW2 to the centrifuge tube (check that anhydrous ethanol has been added before use), vortex for 5 seconds, and then place on a thermostatic mixer at 25° C and 1600 rpm for 2 minutes.
10. Fix the centrifuge tube on a magnetic rack and let it stand for 1 minute, after which the solution is discarded.
11. Repeat steps 9-10.
12. After brief centrifugation of the centrifuge tube, reattach it to the magnetic rack and remove the solution from the bottom of the tube with a pipette, uncap and allow to stand at room temperature for 5-10 minutes to allow the ethanol to evaporate.
13. Add 50-100 μL of RNase-Free Water to the centrifuge tube and vortex to fully suspend the beads in the elution chamber.
The centrifuge tubes were then fixed in a thermostatic mixer at 25° C and 1600 rpm for 10 min to elute.
14. The centrifuge tube is fixed on a magnetic rack and left to stand for 2 minutes. After the Magbeads are completely adsorbed to the sidewalls of the centrifuge tube, transfer the eluate to a new centrifuge tube with a pipette and store at -20℃.

Procedure (4mL plasma as an example)

1. Add reagents to the 24 DW deep well plate according to the table below:

plasma volume	2 mL	4 mL
placement	Reagents and dosage	Reagents and dosage
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. Place the "24 DW Plate and Tips Pack" in the appropriate place in the Nucleic Acid Extractor and run the "CW2560 ctDNA Program".

3. After about 25 minutes, pause the instrument, take the Plate 1 out of the instrument and place it on ice for 5-10 minutes, then add the reagent according to the table.

plasma volume	2 mL	4 mL
placement	Reagents and dosage	Reagents and dosage
Plate 1	Buffer MPL: 2 mL Isopropyl alcohol: 0.5 mL Magbeads ZN: 30 μL	Buffer MPL: 4 mL Isopropyl alcohol: 1 mL Magbeads ZN: 60 μL

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