9. Add 750 μL of BufferGW2 to the adsorbent column (please check whether 100% ethanol is added before use), centrifuge at 12,000 rpm for 30 s, discard the waste solution, and put the adsorbent column back into the collection tube.
10. Add 750 μL of 100% ethanol to the adsorbent column and centrifuge at 12,000 rpm for 30 s. Discard the waste solution, and put the adsorbent column back into the collection tube.
11. Centrifuge at 12,000 rpm for 2 min and discard the waste solution. Leave the adsorption column at room temperature for several minutes to dry thoroughly. 12000 rpm for 30 s. Discard the filtrate and reuse the collection tube.

    Note: The purpose of this step is to remove residual ethanol from the adsorbent column; ethanol residue can interfere with subsequent enzymatic reactions.
12. Place the adsorbent column in a new centrifuge tube, add 20-100 μL of Buffer EBL or RNase-Free water to the middle of the adsorbent column overhanging the column, leave it at room temperature for 2-5 min, centrifuge at 12,000 rpm for 1 min, collect the DNA solution, and store the DNA at -20°C.

    Note: 1) If the downstream experiment is sensitive to pH, you can use RNase-Free water for elution. The pH of the eluent has a great influence on the elution efficiency, if using water as the eluent should ensure that its pH is 7.0-8.5 (you can use NaOH to adjust the pH of water to this range), the elution efficiency is not high when the pH is lower than 7.0.

    2) Preheat the elution buffer Buffer EBL to 60℃ and use it, and incubate it at room temperature for 5 min before centrifugation, which can increase the yield.

    3) If the final concentration of DNA is to be increased, the resulting solution can be reintroduced into the adsorption column and left at room temperature for 2-5 min and centrifuged at 12,000 rpm for 1 min.

    4) Because DNA stored in water is affected by acidic hydrolysis, for long-term storage, elution with BufferEBL and storage at -20°C is recommended.

Version: 12/2023

CWhipro Circulating Nucleic Acid Kit

Cat. No. : CW2603S (50 preps)

Storage Condition : Storage Spin Column DF at 2-8°C for one year. Other components are stored at room temperature (15–30°C) .

Components

Component	CW0535S 50 preps
Buffer CL	45 mL
Buffer CB (concentrate)	60 mL
Buffer GW1 (concentrate)	13 mL
Buffer GW2 (concentrate)	15 mL
Buffer EBL	10 mL
Proteinase K	100 mg
Proteinase K Storage Buffer	5 mL
Spin Columns DF with Collection Tubes	50
Centrifuge Tubes (1.5 mL)	50

Introduction

The CWhipro Circulating Nucleic Acid Kit provides a rapid and easy method for efficient purification of circulating nucleic acids from up to 0.1-1 mL human plasma, serum, urine and other acellular body fluids. Samples can be either fresh or frozen (provided that they have not been frozen and thawed more than once). The CWhipro Circulating Nucleic Acid procedure is based on spin column technology and comprises 4 steps (lyse, bind, wash, elute). The robust procedure helps to eliminate sample-to-sample cross-contamination and increases user safety when handling potentially infectious samples. The kit without the use of organic solvents, and allows for simultaneous processing of single or multiple samples in less than 120 minutes. The Kit also delivers excellent recovery of fragmented nucleic acids as short as 30 bp. Cell-free DNA purified using the kit are free of proteins, nucleases, and other impurities, ready for applications such as PCR, microarrays, and next generation sequencing.

Reagents to Be Supplied by User

100% ethanol
Isopropanol (100%)

Precautions

1. Proteinase K Storage Buffer of 5 mL is added to Proteinase K to dissolve it and stored at -20℃. The prepared Proteinase K should not be kept at room temperature for a long time.
2. Repeated freezing and thawing of the samples should be avoided, as this may lead to a decrease in the amount extracted.
3. This kit can extract 0.1-1 mL of liquid sample.
4. Please check Buffer CL, Buffer CB for crystallization or precipitation before use, if there is any crystallization or precipitation, please re-dissolve Buffer CL, Buffer CB by incubation at 56℃ in a water bath.
5. Before first use isopropanol should be added to Buffer CB according to the instructions on the reagent bottle label, mixed well, and labeled on the reagent bottle label.
6. Before the first use, 100% ethanol should be added to Buffer GW1 and Buffer GW2 according to the instructions on the label of the reagent bottle, mixed well, and labeled on the label of the reagent bottle.

7. Please preheat the water bath to 60°C before starting the experiment.
8. The elution buffer Buffer EBL can be preheated to 60°C and then used.

Protocol

1. Add 20 μL of Proteinase K to the centrifuge tube (self-provided).
2. Add 200 μL of serum or plasma to the tube.

   Note: If the sample volume is greater than 200 μL, increase the amount of Proteinase K, Buffer CL and Buffer CB reagents proportionally as follows.

   Table1. The recommended added volume of buffer for different sample volumes.

Sample volume / Buffer volume	200 μL	300 μL	600 μL	800 μL	1000 μL
Buffer CL	160 μL	240 μL	480 μL	640 μL	800 μL
Buffer CB	360 μL	540 μL	1080 μL	1440 μL	1800 μL
Proteinase K	20 μL	30 μL	60 μL	80 μL	100 μL

3. Add 160 μL Buffer CL. Close the cap and mix thoroughly by pulse-vortexing for 30 s.
4. Incubate at 60°C for 30 min. Mix by inverting or shaking every 10 min during incubation.

   Note: 200 μL serum or plasma samples can be incubated at 60°C for 10-15 min.
5. Add 360 μL Buffer CB (please check whether isopropanol is added before use) to the lysate and vortex at maximum speed for 30 s to mix thoroughly.
6. Ice bath for 5 min and centrifuge briefly to concentrate the solution on the walls and wall caps to the bottom of the tube.
7. Add all of the solution obtained in step 6 to the Spin Columns DF that has been loaded into the collection tube, and if the solution cannot be added all at once, it can be transferred in several times. centrifuge at 12,000 rpm for 1 min, discard the waste solution, and put the adsorbent column back into the collection tube.
8. Add 500 μL of Buffer GW1 to the column (please check whether 100% ethanol is added before use), centrifuge at 12,000 rpm for 30 s, discard the waste solution, and put the column back into the collection tube.