CWhipro Circulating Nucleic Acid Kit

Project number: C665709

Storage conditions: Spin Column DF 2-8° C for 1 year, other components at room temperature (15-30° C).

Product content

Component	C665709-50T
Buffer CL	45ml
Buffer CB (concentrate)	60ml
Buffer GW1 (concentrate)	13ml
Buffer GW2 (concentrate)	15ml
Buffer EBL	10ml
Proteinase K	100mg
Proteinase K Storage Buffer	5ml
Spin Columns DF with Collection Tubes	50
Centrifuge Tubes (L-1.5 ml)	50

Product Introduction

This kit is suitable for the extraction of free DNA from fresh or frozen serum, plasma, lymph fluid and other cell-free body fluids.This kit adopts centrifugal adsorption columns that can specifically bind nucleic acids and a unique buffer system.After the sample is lysed, the free DNA binds to the silica gel membrane under high salt conditions, and the free DNA elutes from the silica gel membrane at low salt and high pH. The product can handle liquid samples of 0.1-1 ml, and the elution volume of the configured high-efficiency micro adsorption column can be as low as 20 μl. The purified DNA is of high yield and quality, with maximum removal of proteins, pigments, lipids, and other inhibitors, and the rate of free DNA yield is highly dependent on the type of samples, storage conditions, time, and inter-individual variations. The quality of free DNA obtained from purification is stable and reliable, and can be directly used in molecular biology experiments such as PCR, fluorescence quantitative PCR and second generation sequencing.

Self-contained reagents: anhydrous ethanol, isopropanol.

Pre-experiment Preparation and Important Notes

Add 5 ml of Proteinase K Storage Buffer to Proteinase K to dissolve it and store it at -20℃. Do not leave the prepared Proteinase K at room temperature for a long time.

Repeated freezing and thawing of the sample should be avoided, as this can lead to a decrease in extraction.

This kit can extract 0.1-1 ml of liquid samples.

Before use, please check Buffer CL, Buffer CB for crystallization or precipitation, if there is any crystallization or precipitation, please re-dissolve Buffer CL, Buffer CB by incubation at 56℃ in a water bath.

Before first use isopropyl alcohol should be added to Buffer CB according to the instructions on the reagent bottle label, mixed well, and labeled on the reagent bottle label.
Before the first use, anhydrous 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.
Preheat the water bath to 60° C before starting the experiment.
The elution buffer Buffer EBL can be preheated to 60° C and used.

Operation steps

Add 20 μl of Proteinase K to the centrifuge tube (supplied).
Add 200 μl of serum/plasma sample.
Note: When the sample volume exceeds 200 μl, please increase the amount of Proteinase K, Buffer CL and Buffer CB reagents in equal proportions, and the specific amount of reagents added can be referred to the attached table.
3. Add 160 μl Buffer CL, mix upside down and shake vigorously for at least 30 seconds.
4. Incubate at 60° C for 30 minutes, during which time mixing was inverted several times.
Note: Incubation of 200μl serum/plasma samples at 60° C for 10-15 minutes is sufficient.
Add 360 μl of Buffer CB (check for addition of isopropanol before use) and shake until thoroughly mixed.
Ice bath for 5 minutes and centrifuge briefly to concentrate the liquid on the walls and wall caps to the bottom of the tube.
Add all of the solution obtained in step 6 to the adsorption columns (Spin Columns DF) that have been loaded into the collection tubes, and if the solution cannot be added all at once, it can be transferred in several times. centrifuge the columns at 12,000 rpm for 1 minute, pour off the waste solution from the collection tubes, and put the columns back into the collection tubes.
Add 500μl of Buffer GW1 to the adsorbent column (check that anhydrous ethanol is added before use),centrifuge the column at 12,000rpm for 30 seconds, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.
Add 750 μl Buffer GW2 to the adsorbent column (check that anhydrous ethanol is added before use), centrifuge at 12,000 rpm for 30 seconds, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.
10. Add 750 μl of anhydrous ethanol to the adsorbent column and centrifuge at 12,000 rpm for 30 s. Pour off the waste liquid in the collection tube and put the adsorbent column back into the collection tube.
11. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.
Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with the subsequent enzymatic reaction.
12. Place the adsorption column in a new centrifuge tube, add 20-100 μl Buffer EBL or sterilized water to the middle part of the adsorption column overhanging the column, leave it at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20℃.

Note: 1) If the downstream experiment is sensitive to pH, you can use sterilized water for elution. The pH value of the eluent has a great influence on the elution efficiency, if water is used as the eluent should ensure that its pH value is 7.0-8.5 (you can use NaOH to adjust the pH value of water to this range), and the elution efficiency is not high when the pH value is lower than 7.0.
2) Preheat the elution buffer BufferEBL to 60℃ and use it, and incubate it at room temperature for 5 minutes before centrifugation to 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 minutes and centrifuged at 12,000 rpm for 1 minute.
(4) Because DNA preserved in water will be affected by acidic hydrolysis, for long-term storage, it is recommended to elute it with Buffer EBL and store it at -20℃.

Table: Recommended reagent additions for different sample sizes

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