CWhipro Circulating DNA Midi Kit

Project number: C665715

Storage conditions: Spin Columns DG 2-8° C, other components room temperature (15-30° C)

Product content

Component	C665715-10T	C665715-50T
Buffer CL	45mL	220mL
Buffer CB (concentrate)	60mL	300mL
Buffer GTL	15mL	60mL
Buffer GW1 (concentrate)	3mL	13mL
Buffer GW2 (concentrate)	3mL	15mL
Buffer EBL	2mL	10mL
Proteinase K	100mg	3×180mg
Proteinase K Storage Buffer	5mL	30mL
Spin Columns DG With Collection Tubes	10	50
Tube Extenders (20mL)	10	50
VacConnectors	10	50
Centrifuge Tubes (L-1.5mL)	10	50

Product Introduction

This kit is suitable for the extraction of free DNA from fresh or frozen serum, plasma, amniotic fluid, urine and other cell-free body fluids.This kit adopts 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. This kit uses the negative pressure method and is also equipped with extension tubes that can handle up to 5 ml of sample. The purified DNA is of high yield and good quality, with maximum removal of proteins, pigments, lipids and other inhibitory impurity contamination. The free DNA obtained from purification is of stable and reliable quality 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

Preparation and important precautions before the experiment

1. Add the specified amount of Proteinase K Storage Buffer to each tube of Proteinase K powder, allow it to dissolve completely, and then store at -20℃. Do not leave the prepared Proteinase K at room temperature for a long time, and avoid repeated freezing and thawing to avoid affecting its activity. The other components can be stored stably for one year in a dry, room temperature (15-30° C) environment. For longer storage, store at 2-8° C.

Cat. No.	C665709-10T	C665709-50T
Proteinase K Storage Buffer	5mL	Add 9mL to each bottle

2. Repeated freezing and thawing of samples should be avoided, as this may result in smaller extracted DNA fragments and reduced extraction.
3. This kit can extract cfDNA from up to 5mL of serum plasma, 4mL of urine.

4. 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.
5. 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.
6. Before use, please check whether Buffer CL, Buffer CB appears to be crystallized or precipitated, if there is any crystallization or precipitation, please re-dissolve Buffer CL, Buffer CB by incubation at 56℃ in a water bath, mix well and then use.
7. The water bath was preheated to 60° C before the start of the experiment.
8. The elution buffer Buffer EBL can be preheated to 60° C.
9. Negative pressure device.

Operation steps

Serum, plasma samples (1-5mL)
1. Sample processing: Add 1mL of serum/plasma sample to the centrifuge tube (self-prepared), if the sample is less than 1mL, add PBS solution to make up to 1mL volume.
Note: When the sample volume exceeds 1mL, please increase the amount of Proteinase K, Buffer CL, and Buffer CB reagents in equal proportion, and the specific amount of reagents to be added can be referred to Exhibit 1.
2. Add 100 μL of Proteinase K to the above solution and mix well.
3. Add 800 μL of Buffer CL and shake vigorously for at least 30 seconds.
4. Incubate at 60° C for 30 min, during which time invert and mix several times.
5. Add 1800 μL of Buffer CB (check for addition of isopropanol before use) and mix upside down 10 times or shake vigorously for 15-30 seconds.
6. Ice bath for 5 minutes.
7. To properly connect the negative pressure unit, insert the connection tubes (VacConnectors) into the sockets of the negative pressure unit.
8. Insert the adsorption column (Spin Column DG) onto the connection tube.
9. Insert the extension tubes (Tube Extenders) into the adsorption column with open caps.
CAUTION: Ensure that the connecting tube, adsorbent column and extension tube are securely connected to prevent leakage.
10. Add all the mixed solution after the ice bath into the extension tube, turn on and adjust the negative pressure to -900~-800mbar, and slowly suck out the solution in the tube. When the solution is completely sucked away, turn off the negative pressure switch and carefully remove the extension tube when the pressure returns to 0mbar.
11. Add 500μL of Buffer GW1 to the column (check whether anhydrous ethanol is added before use), turn on and adjust the negative pressure to -900~-800mbar, wait for the solution to be completely absorbed, and then turn off the negative pressure switch.
12. Add 750μL Buffer GW2 to the adsorption column (check whether anhydrous ethanol is added before use), turn on and adjust the negative pressure to -900~-800mbar, wait until the solution is completely absorbed, and turn off the negative pressure switch.

13. Add 750μL of anhydrous ethanol to the adsorption column, turn on and adjust the negative pressure to -800~-900mbar, wait until the solution is completely absorbed, and turn off the negative pressure switch.
14. When the pressure returns to 0 mbar, remove the column and place it in a new Collection Tube and centrifuge at 12,000 rpm for 3 minutes, pouring off the waste liquid from the tube. Leave the column at room temperature for a few 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.
15. Place the adsorption column in a new 1.5mL centrifuge tube (Centrifuge Tube), add 20-150 μL Buffer EBL to the center of the adsorption column overhanging the center of the adsorption column, let it stand at room temperature for 3 minutes, centrifuge it at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20℃.

Exhibit 1: Recommended amount of reagents to be added for different serum/plasma sample sizes

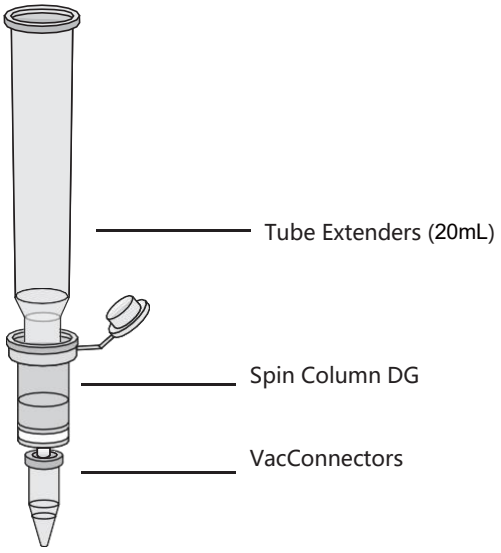
Sample volume / Reagent addition amount	1mL	2mL	3mL	4mL	5mL
Proteinase K	100μL	200μL	300μL	400μL	500μL
Buffer CL	800μL	1.6mL	2.4mL	3.2mL	4mL
Buffer CB	1.8mL	3.6mL	5.4mL	7.2mL	9mL

Urine samples (1-4 ml)
1. Sample handling:
Add 1mL of urine sample to a centrifuge tube (provided). If the sample is less than 1mL, add PBS solution to make up to 1mL volume.
Note: When the sample volume exceeds 1mL, add Proteinase K, Buffer GTL, Buffer CL, and Buffer in equal proportions.
CB reagent dosage, the exact amount of reagent added can be found in Exhibit 2.
2. Add 125μL of Proteinase K to the above solution and mix well.
3. Add 1mL Buffer CL, 250μL Buffer GTL and shake vigorously for at least 30 seconds.
4. Incubate at 60° C for 30 minutes, during which time mixing was inverted several times.
5. Add 3.6mL Buffer CB (check for addition of isopropanol before use) and shake vigorously for 15-30 seconds.
6. Ice bath for 5 minutes.
7. Connect the negative pressure unit correctly by inserting the connection tubes (VacConnectors) into the sockets of the negative pressure unit.
8. Insert the adsorption column (Spin Column DG) onto the connection tube.
9. Insert Tube Extenders into the open-capped adsorption column.
CAUTION: Ensure that the connecting tube, adsorbent column and extension tube are securely connected to prevent leakage.
10. Add all the mixed solution after the ice bath into the extension tube, turn on and adjust the negative pressure to -900~-800mbar, and slowly suck out the solution in the tube. When the solution is completely sucked away, turn off the negative pressure switch and carefully remove the extension tube when the pressure returns to 0mbar.

11. Add 500μL of Buffer GW1 to the column (check whether anhydrous ethanol is added before use), turn on and adjust the negative pressure to -900~-800mbar, wait for the solution to be completely absorbed, and then turn off the negative pressure switch.
12. Add 750μL Buffer GW2 to the adsorption column (check whether anhydrous ethanol is added before use), turn on and adjust the negative pressure to -900~-800mbar, wait until the solution is completely absorbed, and turn off the negative pressure switch.
13. Add 750μL of anhydrous ethanol to the adsorption column, turn on and adjust the negative pressure to -900~-800mbar, wait until the solution is completely absorbed, and turn off the negative pressure switch.
14. When the pressure returns to 0 mbar, remove the column and place it in a new Collection Tube and centrifuge at 12,000 rpm for 3 minutes, pouring off the waste liquid from the tube. Leave the column at room temperature for a few 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.
15. Place the adsorption column in a new 1.5mL centrifuge tube (Centrifuge Tube), add 20-150 μL of Buffer EBL to the center of the adsorption column overhanging the column, let it stand at room temperature for 3 minutes, centrifuge it at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20° C.

Exhibit 2: Recommended amount of reagents to be added for different urine sample sizes

Sample volume / Reagent addition amount	1mL	2mL	3mL	4mL
Proteinase K	125μL	250μL	375μL	500μL
Buffer CL	1mL	2mL	3mL	4mL
Buffer GTL	250μL	500μL	750μL	1mL
Buffer CB	3.6mL	5.4mL	7.2mL	9mL



Connection Diagram of Tube Extenders (20mL), Spin Column DG, VacConnectors