HiPure Circulating DNA Midi Kit C

Introduction

Free-circulating nucleic acids, such as tumor-specific extracellular DNA fragments and mRNAs in the blood or fetal nucleic acids in maternal blood, are present in serum or plasma usually as short fragments, <1000bp(DNA). HiPure Circulating DNA Midi Kit enables efficient purification of these circulating nucleic acids from human plasma, serum, or urine.Samples can be rather fresh or frozen (provided that they have not been frozen and thawed more than once).

Kit Contents

Product	D318200C	D318201C	D318202C
Purification times	4 Preps	10 Preps	50 Preps
Buffer ACL	20 ml	50 ml	250 ml
Buffer ACB*	30 ml	60 ml	300 ml
Buffer DCW1*	4.4 ml	4.4 ml	22 ml
Buffer DCW2*	5 ml	5 ml	10 ml
Proteinase K	50 mg	110 mg	540 mg
Protease Dissolve Buffer	5 ml	10 ml	30 ml
Carrier RNA	110 µg	110 µg	110 µg
Nuclease Free Water	3 ml	10 ml	20 ml
HiPure CFDNA Mini Columns	4	10	50
2 ml Collection Tubes	8	20	100
Extender Tube	4	10	50
Vac-Connector	4	10	50

Storage and Stability

Proteinase K and carrier RNA should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

Materials and Equipment to be Supplied by User

- Add 20ml (4 Preps) or 40ml (10 Preps) or 200ml (50 Preps) Isopropanol to the bottle of Buffer ACB and store at room temperature
- Add 5.6ml (4 Preps) or 5.6ml (10 Preps) or 28ml (50 Preps) 100% ethanol to the bottle of Buffer DCW1 and store at room temperature
- Add 20ml (4 Preps) or 20ml (10 Preps) or 40ml (50 Preps) 100% ethanol to the bottle of Buffer DCW2 and store at room temperature
- Add Protease Dissolve Buffer to Proteinase K to get a final concentration at 20mg/ml and store at -20°C
- Add Nuclease Free Water to Carrier RNA to get a final concentration at 0.2ug/ul and store at -20°C
- 15~50ml centrifuge tubes
- Heat block or water bath capable of 60°C
- 100% ethanol

Reagent volume follow the table:

Sample volumes	1ml	2ml	3ml	4ml	5ml
Proteinase K	100µl	200µl	300µl	400µl	500µl
Buffer ACL	0.8ml	1.6ml	2.4ml	3.2ml	4.0ml
Carrier RNA	5µl	5µl	5µl	5µl	5µl
Buffer ACB	1.8ml	3.6ml	5.4ml	7.2ml	9ml
Buffer DCW1	750ul	750ul	750ul	750ul	750ul
Buffer DCW2	750ul	750ul	750ul	750ul	750ul
100% Ethanol	750ul	750ul	750ul	750ul	750ul

Protocol for 1~5ml serum or plasma

This protocol is for purification of circulating DNA and RNA from 1~5ml of serum or plasma.

1. Pipet • 200µl, or 500µl Proteinase K into a 15~50ml centrifuge tube.

2. Add • 2ml, or 5ml of serum or plasma to the tube, mix thoroughly.

3. Add • 1.6ml , or 4ml Buffer ACL and 5µl of Carrier RNA (1µg) to the tube, Close the cap and mix thoroughly by pulse-vortexing for 30s. Incubate at 60°C for 30min.

4. Add • 3.6ml, or 9ml of Buffer ACB to the lysate in the tube, Close the cap and mix thoroughly by pulse-vortexing for 30s. Incubate the lysate-buffer ACB mixture in the tube for 5min on ice.

5. Connect a new Hipure CFDNA mini column into a new Vac-connector on the vacuum manifold. Insert a new extender tube into the HiPure CFDNA Mini Column.

6. Carefully apply the lysate-Buffer ACB mixture from step 4 into the extender tube of the HiPure CFDNA Mini column.Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the extender tube.

7. Apply 750ul Buffer DCW1 to the column, Leave the lid of the column open,and switch on the vacuum pump. After all of Buffer DCW1 has been drawn through the HiPure CFDNA Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

8. Apply 750ul Buffer DCW2 to the column, Leave the lid of the column open,and switch on the vacuum pump. After all of Buffer DCW2 has been drawn through the HiPure CFDNA Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

9. Apply 750ul Buffer 100% ethanol to the column, Leave the lid of the column open,and switch on the vacuum pump. After all of ethanl has been drawn through the HiPure CFDNA Mini column, switch off the vacuum pump and release the pressure to 0 mbar

10. Close the lid of the HiPure CFDNA Mini Column. Remove it from the vacuum manifold, and discard the VacConnector. Insert the HiPure CFDNA Mini Column into 2ml collection tube. Centrifuge at full speed (13,000 × g) for 3 minute at room temperature.Discard the filtrate and reuse collection tube.

11. Place the HiPure CFDNA Mini column in a new 2ml collection tube.Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

12. **Place the HiPure CFDNA Mini column in a clean 1.5ml collection tube. Carefully apply 30-50µl Nuclease Free Water directly to the center of the column membrane.**Close the lid and incubate at room temperature for 3 minutes

13. Centrifuge at 13,000 × g for 1 minute at room temperature. Store DNA at -20°C.

Troubleshooting Guide

1. Low or no recovery

- **Buffer DCW1/DCW2/ACB did not contain ethanol/Isopropanol:** Ethanol/Isopropanol must be added to Buffer DCW1/DCW2/ACB before used. Repeat procedure with correctly prepare Buffer.
- **Low concentration of target DNA in the Sample:**Samples were standing at room temperature for too long. Repeated freezing and thawing should be avoided. Anticoagulants other than EDTA may lead to accelerated DNA degradation.

2. DNA does not perform well (e.g. in ligation reaction)

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 5 min at room temperature after adding 650ul of Buffer DCW2, then centriufge or Vacuum.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >12,000 x g for 1min, then dry.
- **Inappropriate elution volume used:** Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.

3. Clogged HiPure cfDNA Mini Column

- Vacuum pressure of 800-900 mbar not reached: The vacuum manifold is not tightly closed.
- Transfer the remaining sample lysate to a new tube, place the column in a new collection tube and spin it at full speed for 1 min.