HiPure Circulating Nucleic acid Mini Kit

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 Nucleic Acid Mini Kit enables efficient purification of these circulating nucleic acids from human plasma, serum, or urine. Samples can be fresh or frozen (provided that they have not been frozen and thawed more than once).

Principle

This product is based on silica gel purification. The sample is lysed and digested with lysate and protease, DNA is released into the lysate. Transfer to an adsorption plate and filter column. Nucleic acid is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, Nucleic acid was finally eluted with low-salt buffer (10 Mm Tris, pH 8.0).

Kit Contents

Product	D318101	D318102	D318103
Purification times	20 Preps	50 Preps	250 Preps
Buffer ACL	20 ml	50 ml	250 ml
Buffer ACB*	30 ml	60 ml	300 ml
Buffer DCW1*	13 ml	22 ml	88 ml
Buffer DCW2*	5 ml	10 ml	50 ml
Proteinase K	44 mg	120 mg	540 mg
Protease Dissolve Buffer	5 ml	10 ml	30 ml
Carrier RNA	110 μg	110 μg	310 μg
Nuclease Free Water	3 ml	10 ml	30 ml
HiPure CFDNA Mini Columns	10	50	250
2 ml Collection Tubes	20	100	500

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.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

Materials and Equipment to be Supplied by User

- Add 20ml (20 Preps) or 40ml (50 Preps) or 200ml (250 Preps) Isopropanol to the bottle of Buffer ACB and store at room temperature
- Add 17ml (20 Preps) or 28ml (50 Preps) or 112ml (250 Preps) 100% ethanol to the bottle of Buffer DCW1 and store at room temperature
- Add 20ml (20 Preps) or 40ml (50 Preps) or 200ml (250 Preps) 100% ethanol to the bottle of Buffer DCW2 and store at room temperature
- Add Protease Dissolve Buffer to the Proteinase K to get a final concentration of 20mg/ml and store at -20℃
- Add Nuclease Free Water to the Carrier RNA to get a final concentration of 0.2ug/ul and store at -20℃
- Absolute ethanol
- Heat block or water bath capable of 60°C

Protocol for 1ml serum or plasma

1. Pipet 100μl Proteinase K into a 5 ml microcentrifuge tube.
2. Add 1ml of serum or plasma to the tube, mix for 5s.
3. Add 0.8 ml Buffer ACL and 5μl of Carrier RNA (1μg) to the tube, Close the cap and mix thoroughly by pulse-vortexing for 15s. Incubate at 60℃ for 30min.
4. Add 1.8 ml 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. Insert a HiPure CFDNA Mini Column in a 2ml Collection Tube.
6. Add up to 750μl solution from Step 4 to the Column. Centrifuge at 8,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
7. Repeat Step 6 until all of the sample has been transferred to the column.Discard the filtrate and the collection tube.
8. Insert the column in a new 2ml Collection Tube. Add 650μl Buffer DCW1to the column. Centrifuge at 12,000 × g for 1 minute.Discard the filtrate and reuse collection tube.
9. Add 650μl Buffer DCW2 to the column. Centrifuge at 12,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.
10. Add 650μl Absolute ethanol to the column. Centrifuge at 12,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.
11. Centrifuge the empty column at 12,000 × g for 1 minute at room temperature to dry the column matrix.
12. Place the HiPure CFDNA Mini column to a clean 1.5ml microcentrifuge tube. Open the lid, and incubate the assembly at 56℃ for 10 min to dry the membrane completely.
13. Add 30-50μl Nuclease Free Water directly to the center of the column membrane.Let sit at room temperature for 3 minutes. Centrifuge at 12,000 × g for 1 minute at room temperature.

    Ensure that Nuclease Free Water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50μl), dispense water onto the center of the membrane to ensure complete elution of bound DNA.HiPure CFDNA columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream

application. Remember that the volume of eluate will be up to 5 µl less than the volume of the solution applied to the column. For Elute DNA more thoroughly, repeat step 13 using another volume of water, or using the eluate from step 13.

14. Store DNA at -20℃.

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 800-900mbar 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.