【Product Name】HiPure Circulating DNA Spin Kit D (Spin Protocol)

【Product specifications】50 Preps/Kit

【Intended Use】

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.The extracted products can be used for clinical in vitro detection.

【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).

【Main Composition】

Cat.No	D318203D	Contents
Purification Times	50 Preps	-
Buffer ACL	250 ml	Tris/EDTA/Guanidine Salt
Buffer ACB*	300 ml	Tris/EDTA/Guanidine Salt
Buffer DCW1*	22 ml	Tris/EDTA/Guanidine Salt
Buffer DCW2*	10 ml	Tris/Nacl
Proteinase K	540 mg	10mm Tris,pH8.0
Protease Dissolve Buffer	30 ml	Glycerol/Tris/CaCl2
Carrier RNA	110 μg	Poly A
Nuclease Free Water	20 ml	Water
HiPure CFDNA Mini Columns	50	Silicon Column
2 ml Collection Tubes	100	PP Column
Extender Tube	50	PP Tubes
Support Tubes	50	PP Tubes
Sealing O-Ringes	50	Slicone sealed gasket
50ml Centrifuge Tubes	50	PP Tubes

【Storage conditions and Validity】

Proteinase K, 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.

【Preparation before Use】

- Add 200 ml Isopropanol to the bottle of Buffer ACB, and store at room temperature.
- Add 28 ml absolute ethanol to the bottle of Buffer DCW1, and store at room temperature.
- Add 40 ml absolute ethanol to the bottle of Buffer DCW2, and store at room temperature.
- Add 27ml Protease Dissolve Buffer to the bottle of proteinase K,store at -20~8°C after dissolve.
- Add 0.55ml Nuclease Free Water to the bottle of carrier RNA, store at -20°C after dissolve.

【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】

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℃ 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 column with Sealing O-Ringes, Support Tube and Extender Tubes. Place it into a new 50ml centrifuge tubes.
6. Carefully apply 15ml of the lysate-Buffer ACB mixture from step 5 into the extender tube of the HiPure CFDNA Mini column. Centrifuge at 2000 x g for 3min at room temperature.
7. Remove the column, discard the filtrate, and place the column back into the 50 ml centrifuge tube. Load the remainder of the solution from step 4 onto the column. Close the cap and centrifuge again at 2,000 x g for 3 min.
8. Place the HiPure CFDNA DNA Column into a new 2ml Collection Tubes. Discard the filtrate and extender tube, support tube and 50ml centrifuge tube.
9. Add 650μl Buffer DCW1. Close the cap and centrifuge at 12,000 x g for 1 min.
10. Discard the flow through and reuse the collection Tubes. Add 650μl Buffer DCW2. Close the cap and centrifuge at 12,000 x g for 1 min.
11. Discard the flow through and reuse the collection Tubes. Add 650μl absolute ethanol. Close the cap and centrifuge at 12,000 x g for 1 min.
12. Discard the flow through and reuse the collection Tubes. Centrifuge at 12,000 x g for 1 min. This step helps to eliminate the chance of possible Buffer GW2 carryover.
13. Place the HiPure CFDNA Mini column in a new 2ml collection tube.Open the lid, and incubate the assembly at 56℃ for 10 min to dry the membrane completely.
14. 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.
15. Centrifuge at 13,000 × g for 1 minute at room temperature. 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 800-900 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.