【Product Name】HiPure Circulating DNA Kit

【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 column 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.

【Main Composition】

Cat.No	IVD3182	Contents
Buffer ACL	250 ml	Glycerol/Tris/CaCl2
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 Column
Vac-Connector	50	PP Column

【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 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

【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℃ after dissolve.
- Add 0.55ml Nuclease Free Water to the bottle of carrier RNA, store at -20℃ 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 ø 500μl Proteinase K into a 15~50ml centrifuge tube.**
2. **Add • 2ml, or ø 5ml of serum or plasma to the tube, mix thoroughly.**
3. **Add • 1.6ml , or ø 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 ø 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℃ 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℃.

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.

【Basic Information】

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【Explanation of Marks】

IVD	The product is used in vitro, please don't swallow		Please don't reuse it
	Validity		Please read the instruction book carefully before using
	Warning, please refer to the instructions in the annex		Manufacturer
8℃ 2℃	Temperature scope within which the product is reserve	LOT	Batch number
EC REP	European union authorization representativ		Keep dry
	Avoid overexposure to the sun		Don't use the product when the package is damaged
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		