HiPure Circulating Nucleic acid Micro Kit

Introduction

Free-circulating nucleic acids, such as tumor-specific extracellular Nucleic Acid fragments and mRNAs in the blood or fetal nucleic acids in maternal blood, are present in serum or plasma usually as short fragments, <1000bp(Nucleic Acid). The HiPure Circulating Nucleic Acid Kit enables efficient purification of these circulating nucleic acids from human plasma, serum, or urine. Samples can be either fresh or frozen (provided that they have not been frozen and thawed more than once). Free-circulating cell-free DNA, RNA or viral nucleic acids are eluted in Nuclease Free Water, ready for use in amplification reactions or storage at –30 to –15°C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

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.

Kit Contents

Product Number	D318002	D318003
Purification Times	50 Preps	250 Preps
Buffer ACL	40 ml	200 ml
Buffer DCW1*	22 ml	110 ml
Buffer DCW2*	20 ml	2 x 50 ml
Nuclease Free Water	10 ml	30 ml
Carrier RNA	110 µg	310 µg
Proteinase K	34 mg	180 mg
Protease Dissolve Buffer	1.8 ml	10 ml
HiPure CFDNA Mini Columns	50	250
2ml Collection Tube	100	5 x 100

Storage and Stability

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.

Materials and Equipment to be Supplied by User

- Add 28 ml (50 Preps) or 140 ml (250 Preps) absolute ethanol to the bottle of Buffer DCW1, and store at room temperature.
- Add 80 (50 Preps) or 2 x 200 ml (250 Preps) absolute ethanol to the bottle of Buffer DCW2, and store at room temperature.
- Add 1.7 ml (50 Preps) or 9.0ml (250 Preps) Protease Dissolve Buffer to the bottle of proteinase K, store at -20~8°C after dissolve.
- Add 0.55 (50 Preps) or 1.5ml (250 Preps) Nuclease Free Water to the bottle of carrier RNA, store at -20°C after dissolve.

Protocol for 0.6ml serum or plasma

1. Pipet 30 µl Proteinase K into a 2ml centrifuge tube.

2. Add 0.6 ml of serum or plasma to the tube, mix thoroughly.
   To obtain cell-free nucleic acids from urine or plasma, it is recommended to centrifuge the samples at high speed (e.g., 16,000 x g) for 10 min at 4°C and only use the supernatant for nucleic acid extraction. This will remove cellular material and cellular nucleic acids from the sample.

3. Add 0.6 ml Buffer ACL and 5µl of Carrier RNA (1µg) to the tube, Close the cap and mix thoroughly by pulse-vortexing for 15s.
   If the sample volume is larger than 600µl, increase the amount of Proteinase K and Buffer ACL proportionally. Do not add Proteinase K directly to Buffer ACL. Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.

4. Incubate at 55°C for 15min.

5. **Add 0.3ml Isopropanol to the lysate in the tube, Close the cap and mix thoroughly by pulse-vortexing for 30s.** Incubate for 3min at room temperature.

6. Insert a HiPure CFDNA Mini Column in a 2ml Collection Tube.

7. **Add up to 750µl solution from Step 5 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

8. Repeat Step 7 until all of the sample has been transferred to the column.Discard the filtrate and the collection tube.

9. **Insert the column in a new 2ml Collection Tube. Add 600µl Buffer DCW1to the column.** Centrifuge at 12,000 × g for 1 minute.Discard the filtrate and reuse collection tube.

10. **Add 600µl Buffer DCW2 (Diluted with 100% ethanol prior to use) to the column.** Centrifuge at 12,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.

11. Repeat Steps 10 for a second Buffer DCW2 wash step.

12. Centrifuge the empty column at 12,000 × g for 2 minute at room temperature to dry the column matrix.

13. **Place the column to a clean 1.5ml microcentrifuge tube.** Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

14. **Add 20-100µl 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. Store Nucleic Acid at -20°C.

    Ensure that Nuclease Free Water is equilibrated to room temperature. If elution is done in small volumes (<50µl), the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA. Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will be up to 5µl less than the elution volume applied to the Mini column.

Troubleshooting Guide

1. Low or no recovery

- **Buffer DCW1/DCW2 did not contain ethanol:** Ethanol must be added to Buffer DCW1/DCW2 before used. Repeat procedure with correctly prepare Buffer.
- **Low concentration of target Nucleic Acid 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 Nucleic Acid degradation.
- **Inefficient sample lysis in Buffer ACL:** If Proteinase K was subjected to elevated temperature for a prolonged time, it can lose activity. Repeat the procedure using new samples and fresh Proteinase K.
- **Buffer ACL–carrier RNA mixture not sufficiently mixed：** Mix Buffer ACL with carrier RNA by gently inverting the tube of Buffer ACL– carrier RNA at least 10 times.

2. Nucleic Acid 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 600ul of Buffer DCW2, then centriufge.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at 13,000 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.
- **Interference due to carrier RNA:** If the presence of carrier RNA in the eluate interferes with the downstream enzymatic reaction, it may be necessary to reduce the amount of carrier RNA or to omit it altogether.
- **Buffers not mixed thoroughly：** Salt and ethanol components of wash Buffer DCW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.