HiPure Circulating DNA/RNA Kit

Introduction

This kit provides a simple and fast solution for the extraction of circulating nuclear acid from serum, plasma, and other cell-free liquid samples. Circulating nucleic acid refers to the free extracellular nucleic acid produced by cell apoptosis, of which fragments are generally below 1KB. The kit is based on silica gel column purification technology, which is no need for toxic phenol chloroform extraction and time-consuming alcohol precipitation during the extraction. The obtained Circulating Nucleic Acid can be directly used for quantitative PCR, liquid or solid phase chip analysis, hybridization, and SNP detection.

HiPure Circulating DNA/RNA Kit adopts a unique solution system and multiple layers of filter membranes with different pore sizes, which can efficiently process large volumes of serum and plasma samples and capture extremely small amounts of free nucleic acids.

Kit Contents

Product	R431601	R431602	R431603
Preparation Times	10	50	250
Buffer CFL	30 ml	150 ml	2 x 375 ml
Buffer CFP	5 ml	30 ml	150 ml
Buffer MGW1*	20 ml	100 ml	2 x 250 ml
Buffer RW2*	20 ml	2 x 50 ml	5 x 100 ml
RNase Free Water	5 ml	10 ml	50 ml
HiPure RNA Micro Columns	10	50	5 x 50 ml
HiPure Viral Midi Columns	10	50	5 x 50
15 ml Collection Tubes	10	50	5 x 50
2 ml Collection Tubes	10	50	5 x 50

Storage and Stability

The kit components can be stored 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

- Absolute ethanol (96~100%)
- Isopropanol
- Glacial acetic acid
- Dilute Buffer RW2 with 80ml (10 Preps), 2 x 200 ml (50 Preps) or5 x 400ml (250 Preps) 100% ethanol and store at room temperature
- Dilute Buffer MGW1 with 20ml (10 Preps), 100ml (50 Preps) or 2 x 250ml (250 Preps) Isopropanol and store at room temperature
- Microcentrifuge capable of at least 12,000 × g

Protocol 1. Extract Circulating DNA/RNA from 1~5ml samples

This protocol is suitable for extraction of circulating DNA/RNA, including miRNA, from 1-5ml serum, plasma, or other cell-free liquid samples directly.

1. Centrifuge at 1,900 x g for 10 minutes at 4 °C to separate plasma or serum, transfer the plasma or serum to a new centrifuge tube.
2. Centrifuge at 4,000~5,000 x g for 15 minutes at 4°C to further remove impurities such as cell debris, transfer 1-5 ml supernatant to a new centrifuge tube.
3. **Add 300 µl Buffer CFL to the sample per 1 ml plasma or serum**, vortex to mix well, place at room temperature for 10-15 minutes.

   Optional: Add 30 µl Proteinase K per 1ml plasma or serum samples, mix by turning up and down, incubate at 37 °C for 30 minutes can improve DNA production.
4. **Add 100 µl Buffer CFP to the sample per 1ml plasma or serum**, vortex at high speed for more than 20 seconds, and place on ice for 3 minutes.
   This step will generate a large amount of sediment, fully vortex to disperse to prevent the nucleic acid from being wrapped together by the sediment and losing yield.

5. Centrifuge at 13,000 x g for 5 minutes or Centrifuge at 4,000-5,000 x g for 15 minutes.

6. **Transfer the supernatant to a new centrifuge tube, add equal volume pre-cooled isopropanol (2% glacial acetic acid) to the supernatant, and vortex for 15 seconds.**

7. Insert a HiPure Viral Midi Column to a 15 ml centrifuge tube.

The following centrifugation is carried out at room temperature.

8. **Transfer <4 ml mixture to the column**, centrifuge at 4,000-5,000 x g for 3 minutes.

9. Discard the filtrate and insert the column back into the collection tube. Repeat step 8 until all the mixture is transferred to the column, centrifuge at 4,000-5,000 x g for 3 minutes.

10. Discard the filtrate and insert the column back into the collection tube. **Add 3ml Buffer MGW1 to the column.** Centrifuge at 4,000~5,000 x g for 3 minutes.

11. Discard the filtrate and insert the column back into the collection tube. **Add 3ml Buffer RW2 to the column.** Centrifuge at 4,000~5,000 x g for 15 minutes.

12. Transfer the column to a new 15 ml centrifuge tube. **Add 400 µl RNase Free Water to the membrane center of the column**. Place for 5 minutes, centrifuge at 4,000-5,000 x g for 3 minutes.

13. **Add 200 µl Buffer CFL and 0.9 ml absolute ethanol to the eluent, vortex for 10 seconds.**

14. **Insert a HiPure RNA Micro Column into a 2 ml collection tube. Transfer ≤750 µl mixture to the column,** centrifuge at 12,000 x g for 1 minute.

15. Discard the filtrate and insert the column back into the collection tube. Transfer the remaining mixture to the column, centrifuge at 12,000 x g for 1 minute.

16. **Discard the filtrate and insert the column back into the collection tube. Add 600µl Buffer RW2 to the column.** Centrifuge at 12,000 x g for 30~60 seconds.

    Buffer RW2 must be diluted with absolute ethanol according to the instructions on the bottle label before use.

17. **Repeat Step. 16 once.**

18. Discard the filtrate and insert the column back into the collection tube. Centrifuge at 12,000 x g for 3 minutes.

19. Open the lid of the column and place at room temperature for 10 minutes to dry the column.

20. Transfer the column to a 1.5ml centrifuge tube. **Add 20-50 µl RNase Free Water to the center of the column membrane.** Place at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 minute.

21. Discard the column and store RNA at -20 °C or -80 °C.

Protocol 2. Extract Circulating RNA from 1~5ml samples

This protocol is suitable for extraction of circulating RNA, including miRNA, from 1-5ml serum, plasma, or other cell-free liquid samples directly.

1. Centrifuge at 1,900 x g for 10 minutes at 4 °C to separate plasma or serum, transfer the plasma or serum to a new centrifuge tube.

2. Centrifuge at 4,000~5,000 x g for 15 minutes at 4 °C to further remove impurities such as cell debris, transfer 1-5 ml supernatant to a new centrifuge tube.

3. **Add 300 µl Buffer CFL to the sample per 1 ml plasma or serum**, vortex to mix well, place at room temperature for 10-15 minutes.

4. **Add 100 µl Buffer CFP to the sample per 1ml plasma or serum**, vortex at high speed for more than 20 seconds, and place on ice for 3 minutes.
   This step will generate a large amount of sediment, fully vortex to disperse to prevent the nucleic acid from being wrapped together by the sediment and losing yield.

5. Centrifuge at 13,000 x g for 5 minutes or Centrifuge at 4,000-5,000 x g for 15 minutes.

6. **Transfer the supernatant to a new centrifuge tube, add equal volume pre-cooled isopropanol (2% glacial acetic acid) to the supernatant, and vortex for 15 seconds.**

7. Insert a HiPure Viral Midi Column to a 15 ml centrifuge tube.

The following centrifugation is carried out at room temperature.

8. **Transfer <4 ml mixture to the column**, centrifuge at 4,000-5,000 x g for 3 minutes.

9. Discard the filtrate and insert the column back into the collection tube. Repeat step 8 until all the mixture is transferred to the column, centrifuge at 4,000-5,000 x g for 3 minutes.

10. Discard the filtrate and insert the column back into the collection tube. **Add 3ml Buffer MGW1 to the column.** Centrifuge at 4,000~5,000 x g for 3 minutes.

11. Discard the filtrate and insert the column back into the collection tube. **Add 3ml Buffer RW2 to the column.** Centrifuge at 4,000~5,000 x g for 10 minutes.

12. Transfer the column to a new 15 ml centrifuge tube. **Add 600 µl Buffer CFL to the membrane center of the column**. Place for 5 minutes, centrifuge at 4,000-5,000 x g for 3 minutes.

13. **Add 0.9 ml absolute ethanol to the eluent, vortex for 10 seconds.**

14. **Insert a HiPure RNA Micro Column into a 2 ml collection tube. Transfer ≤750 µl mixture to the column,** centrifuge at 12,000 x g for 1 minute.

15. Discard the filtrate and insert the column back into the collection tube. Transfer the remaining mixture to the column, centrifuge at 12,000 x g for 1 minute.

16. **Discard the filtrate and insert the column back into the collection tube. Add 600µl Buffer RW2** to the column. Centrifuge at 12,000 x g for 30~60 seconds.
    Buffer RW2 must be diluted with absolute ethanol according to the instructions on the bottle label before use.

17. **Repeat Step. 16 once.**

18. Discard the filtrate and insert the column back into the collection tube. Centrifuge at 12,000 x g for 3 minutes.

19. Open the lid of the column and place at room temperature for 10 minutes to dry the column.

20. Transfer the column to a 1.5ml centrifuge tube. **Add 20-50 µl RNase Free Water to the center of the column membrane.** Place at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 minute.

21. Discard the column and store RNA at -20 °C or -80 °C.

Troubleshooting Guide

Low yield Nucleic Acid

- **Not enough sample amount**: cell-free DNA/RNA is very amount in plasma/serum samples. Increase the sample amount for extraction.
- **Not add EDTA as Anticoagulants**: blood sample should be stored in cell free nucleic acid preserve tube or add EDTA as anticoagulants. Cell free nucleic acid is easy to be degradation in storage.
- **Sample was feezed thawing more than once**: cell free nucleic acid is easy to b degradation. Samples can not be reused after thawing.
- **Column clogging**: samples not mixed throughly after adding Buffer CFP.
- **Insufficient elution process**:: Increase elution buffer (RNase Free Water) volumn, place the column at room temperature for 3 minutes
- **Buffer MGW1 or Buffer RW2 not prepare correctly**:: Buffer MGW1/ Buffer RW2 should add isopropanol/ethanol following the label before use.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the column membrane by centrifuged at >12,000 x g for 3 min.