

HiPure Circulating DNA/RNA Kit

Introduction

The HiPure Circulating DNA/RNA Kit is designed for isolation of cell-free nucleic acids from 1~5 ml serum, plasma, and other cell-free liquid samples. The procedure uses metal salt-mediated selective protein precipitation to remove proteins and other sample-derived impurities, followed by silica membrane purification. No phenol-chloroform extraction is required. The purified nucleic acids are suitable for downstream applications such as quantitative PCR/RT-PCR, microarray analysis, hybridization, NGS and SNP detection.

The kit provides two alternative protocols. Protocol 1 enables the co-purification of cell-free DNA and RNA, including miRNA. Protocol 2 incorporates DNase digestion to remove DNA and isolate cell-free RNA, including miRNA.

The kit employs a two-stage spin-column procedure. Cell-free nucleic acids are first captured from the processed sample using a large-capacity column and subsequently concentrated using a micro column. This procedure allows cell-free nucleic acids from medium-volume samples to be eluted in a small volume.

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	250
HiPure Viral Midi Columns	10	50	250
15 ml Collection Tubes	10	50	250
2 ml Collection Tubes	10	50	250

Storage and Stability

The kit components can be stored at room temperature (15–25°C) and are stable for at least 24 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) or 5 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 × 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 × 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 yield.
4. **Add 100 µl Buffer CFP to the sample as per 1 ml 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 × g for 5 minutes or Centrifuge at 4,000-5,000 × g for 15 minutes

at room temperature.

6. **Transfer the supernatant to a new centrifuge tube, add equal volume ice-cooled isopropanol (2% glacial acetic acid) to the supernatant, and vortex for 15 seconds.**
- **Isopropanol preparation:** add 9.8ml isopropanol to a 15ml centrifuge tube, add 0.2ml glacial acetic acid to the tube, mix well and incubate on ice before use.
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. Discard the filtrate and insert the column back into the collection tube.
9. **Repeat step 8 until all the mixture is transferred to the column.**
10. **Add 3ml Buffer MGW1 to the column.** Centrifuge at 4,000~5,000 x g for 3 minutes. Discard the filtrate and insert the column back into the collection tube.
11. **Add 3ml Buffer RW2 to the column.** Centrifuge at 4,000~5,000 x g for 15 minutes. Discard the filtrate and transfer the column to a new 15 ml centrifuge tube.
12. **Add 600 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. Discard the column.
13. **Add 250 µl Buffer CFL and 0.75 ml isopropanol 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. Discard the filtrate and insert the column back into the collection tube.
15. **Transfer the remaining mixture to the column,** centrifuge at 12,000 x g for 1 minute. Discard the filtrate and insert the column back into the collection tube.
16. **Add 500µl Buffer RW2 (diluted with ethanol) to the column.** Centrifuge at 12,000 x g for 30~60 seconds. Discard the filtrate and insert the column back into the collection tube.
17. **Repeat Step. 16 once.**
18. **Centrifuge at 12,000 x g for 3 minutes.**
19. **Take out the column from collection tube, open the lid of the column and place at room temperature for 10 minutes to dry the column.**

20. Transfer the column to a new 1.5ml centrifuge tube. **Add 15-50 μ l RNase Free Water to the center of the column membrane.** Place at room temperature for 2 minutes. Centrifuge at 12,000 $\times$ g for 1 minute.
21. Discard the column and store cDNA/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 $\times$ 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 $\times$ 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 μ l 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 μ l Buffer CFP to the sample per 1 ml 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 $\times$ g for 5 minutes or Centrifuge at 4,000-5,000 $\times$ 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 1.5 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 $\times$ g for 3 minutes. Discard the filtrate and insert the column back into the collection tube.
9. Repeat step 8 until all the mixture is transferred to the column.
10. **Add 3ml Buffer MGW1 to the column.** Centrifuge at 4,000~5,000 $\times$ g for 3 minutes. Discard the filtrate and insert the column back into the collection tube.
11. **Add 3ml Buffer RW2 to the column.** Centrifuge at 4,000~5,000 $\times$ g for 10 minutes.

Discard the filtrate and transfer the column to a new 15 ml centrifuge tube.

12. **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. Discard column.
13. **Add 0.9 ml absolute ethanol to the mixture and 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. Discard the filtrate and insert the column back into the collection tube.
15. Transfer the remaining mixture to the column, centrifuge at 12,000 x g for 1 minute. Discard the filtrate and insert the column back into the collection tube.
16. (Optional: remove DNA thoroughly)

16.1 **Prepare DNase I mixture in a new microcentrifuge tube on following table.** This kit not provided, customer can purchase DNase I Set B (Magen Cat# C12138) separately.

Reagent	Volume
DNase Buffer	70 µl
DNase I (10 units/µl)	10 µl

16.2 Apply the DNase I mixture to the centre of the column membrane, and incubate at room temperature (15- 30°C) for 15 min.

17. **Add 600µl Buffer MGW1 to the column, stay at room temperature for 1 min. Centrifuge at 12,000 x g for 1 min.**
18. **Insert the column into a new 2ml Collection Tube, transfer the filtrate back to the column. Centrifuge at 12,000 x g for 1 min.** Discard the filtrate and insert the column back into collection tube.
19. **Add 600µl Buffer RW2 (diluted with ethanol) to the column.** Centrifuge at 12,000 x g for 30~60 seconds. Discard the filtrate and insert the column back into the collection tube.
20. Repeat Step 19 once.
21. Centrifuge at 12,000 x g for 3 minutes to dry the column matrix.
22. Open the column lid, stay the column at room temperature for 10 min to dry the column matrix thoroughly.

23. Transfer the column to a 1.5ml centrifuge tube. **Add 20-50 μ l RNase Free Water to the center of the column membrane.** Place at room temperature for 2 minutes. Centrifuge at $12,000 \times g$ for 1 minute.
24. Discard the column and store cRNA at $-20\text{ }^{\circ}\text{C}$ or $-80\text{ }^{\circ}\text{C}$.

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 freezed 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 thoroughly 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.
- **Euate contains residual ethanol:** Ensure that the wash flow-through is drained from the column membrane by centrifuged at $>12,000 \times g$ for 3 min.