VAMNE MagUltra Circulating Cell-free DNA Isolation Kit

N913

Vazyme

Instruction for Use

Version 25.2

Vazyme

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

VAMNE MagUltra Circulating Cell-free DNA Isolation Kit is based on superparamagnetic nanoparticle purification technology. It is suitable for purifying cell-free DNA (cfDNA) from 0.2 - 10 ml serum, 0.2 - 10 ml plasma, 2 - 10 ml urine, or other cell-free body fluid samples efficiently. This reagent is especially optimized for the recovery of low molecular weight nucleic acids, with high extraction efficiency, good quality and reliable reproducibility. cfDNA can be simply and rapidly extracted to apply in quantitative PCR, NGS or other downstream experiments. This kit is compatible with magnetic rod-based automated nucleic acid extraction instrument for high-throughput extraction.

02/Components

	Components	N913-01 (50 rxns)	N913-02 (100 rxns)
BOX 1	Proteinase K	1.5 ml	2 × 1.5 ml
	MagUltra Beads B	1.5 ml	2 × 1.5 ml
BOX 2	Buffer ES	5 ml	10 ml
	Buffer L/B	125 ml	2 × 125 ml
	Buffer WA	100 ml	200 ml
	Elution Buffer	5 ml	10 ml

▲ Number of preps is calculated based on 2 ml sample input per reaction.

03/Storage

BOX 1: Store at 2 ~ 8°C and ship on ice pack.
BOX 2: Store at 15 ~ 25°C and ship at room temperature.

04/Applications

Serum, plasma, urine and other cell-free body fluid samples.

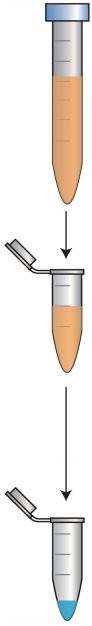
05/Self-prepared Materials

1. 80% ethanol is needed to be prepared freshly before use. Additional Isopropanol is needed for urine sample extraction.
2. 60°C water bath or metal heating block.
3. Vortex oscillator (suitable for 1.5 ml/15 ml/50 ml tubes).
4. DNase-free low adsorption centrifuge tubes (1.5 ml/15 ml/50 ml) and the corresponding magnetic stand.

06/Notes

1. For plasma samples in cell-free nucleic acid storage tube, the rapid procedure is not recommended.
2. The MagUltra Beads B needs to be equilibrated at room temperature for 15 min and vortexed thoroughly before use. The MagUltra Beads B needs to be prevented from freezing or centrifugation to avoid irreversible damage.
3. Check Buffer ES, Buffer L/B, and Buffer WA for any precipitate before use. The precipitate can be dissolved in a 50°C water bath. Mix well before use.
4. Repeated freezing and thawing is forbidden for serum/plasma samples, as it may lead to reduced cfDNA yield and residual genomic DNA.
5. The residual ethanol needs to be fully dried in the air-dry step to avoid affecting the downstream experiments. The excessive drying is also forbidden to ensure elution efficiency. No reflection on the beads surface represents a suitable dry condition.
6. To set the automatic extraction process, please refer to automated nucleic acid extraction instruments.
7. If the supplied Buffer WA is insufficient, please separately purchase the Buffer WA (Vazyme #N913-P1).

07/Mechanism & Workflow



Step 1: Sample lysis and cfDNA binding

◇ De-crosslinking: Sequentially add Buffer ES, sample, and Proteinase K solution to the centrifuge tube (do not directly add Proteinase K solution to Buffer ES), and vortex for 10 sec. Incubate the tube in a 60°C water bath for 20 min, and then cool on ice for 5 min.

◇ Lysis and binding: Add Buffer L/B and MagUltra Beads B to the tube, and vortex at room temperature for 10 min.

Step 2: cfDNA rinsing

◇ Remove proteins: Add Buffer WA and vortex for 30 sec to rinse. Then place the tube on a magnetic rack for 2 min and discard the supernatant completely. Repeat this step twice.

◇ Remove salt ions: Add 80% ethanol and vortex for 30 sec to rinse. Then place the tube on a magnetic rack for 2 min and discard the supernatant completely. Repeat this step twice.

◇ Remove ethanol: Air dry at room temperature for 3 - 5 min.

Step 3: cfDNA elution

◇ Add Elution Buffer and vortex at room temperature for 5 min to elute cfDNA.

Fig 1. Workflow of VAMNE MagUltra Circulating Cell-free DNA Isolation Kit

08/Experiment Process

08-1/Sample Preparation

◇ Plasma sample

Centrifuge blood sample at 4,500 rpm (2,000 × g) and 4°C for 10 min. Then transfer the supernatant to a new centrifuge tube. Centrifuge again at 12,000 rpm (16,000 × g) for 10 min or 7,500 rpm (6,000 × g) for 30 min at 4°C. Collect the supernatant and transfer it to a new centrifuge tube.

◇ Serum sample

Incubate the anticoagulant-free blood sample at room temperature for 30 - 45 min for coagulation. Then centrifuge the sample at 2,360 rpm (1,000 × g) and 4°C for 10 - 15 min and transfer the supernatant to a new centrifuge tube. Centrifuge again at 2,360 rpm (1,000 × g) and 4°C for 10 min. Collect the supernatant and transfer it to a new centrifuge tube.

◇ Urine sample

Centrifuge urine sample at 12,000 rpm (16,000 × g) and 4°C for 10 min. Transfer the supernatant to a new centrifuge tube.

For urine sample extraction, please refer to 08-4/High Concentration cfDNA Extraction Process.

For plasma and serum sample extraction, based on the sample volume and output concentration requirement, can choose from 08-2/Large Volume Sample (1 - 10 ml) cfDNA Extraction Procedure, 08-3/Small Volume Sample (200 - 800 µl) cfDNA Extraction Procedure and 08-4/High Concentration cfDNA Extraction Process.

For plasma not separated from Streck Cell-Free DNA BCT only, 08-5/Rapid Extraction Process is available to obtain cfDNA in shorter time but with lower yield.

08-2/Large Volume Sample (1 - 10 ml) cfDNA Extraction Procedure

Reagent preparation

1. Equilibrate the MagUltra Beads B at room temperature for 15 min, and mix thoroughly before use.

2. 80% ethanol (2 ml for each sample).

3. The adding volume of each component for different input volume is referred to Table 1:

Table 1. Reagent Preparation Worksheet for 1 - 10 ml Sample Input Volume

Components	Sample Volume			
	1 ml	2 ml	4 ml	10 ml
Buffer ES	50 µl	100 µl	200 µl	500 µl
Proteinase K	15 µl	30 µl	60 µl	150 µl
Buffer L/B	1.25 ml	2.5 ml	5 ml	12.5 ml
MagUltra Beads B	15 µl	30 µl	60 µl	150 µl
Buffer WA			2 × 1 ml	
80% ethanol			2 × 1 ml	
Elution Buffer	20 µl	30 µl	60 µl	150 µl

Operation steps

Step 1: Sample lysis and cfDNA binding

This process takes **2 ml** sample volume input as an example. The adding volume of each reagent is highlighted in bold and needs to be changed under other condions. The adding volume of each component for other sample input is referred to Table 1.

1. Sequentially add **100 µl** Buffer ES, **2 ml** sample, and **30 µl** Proteinase K to a 15 ml centrifuge tube (do not directly add the Proteinase K solution to Buffer ES), and vortex for 10 sec.
2. Incubate the tube at 60°C for 20 min, then cool on ice for 5 min.
3. Sequentially add **2.5 ml** Buffer L/B and **30 µl** MagUltra Beads B to the centrifuge tube and mix thoroughly.
   ▲ If having multiple samples, premix corresponding reagents based on the ratio of 30 µl MagUltra Beads B per 2.5 ml Buffer L/B for each sample.
4. (Optional) Add 200 ng - 1 µg Carrier RNA (which is self-prepared and needs to be confirmed that it will not impact the downstream experiments).
5. Invert 10 times, and vortex at room temperature for 10 min. Collect the residual liquid by brief centrifugation.
   ▲ Inadequate oscillation may lead to low yield, so continuous vortexing (maintain the liquid in a vortexing state) is required.

Step 2: cfDNA rinsing

1. Load the 15 ml centrifuge tube to the magnetic rack for 5 - 10 min or until the solution becomes clear. Carefully aspirate the supernatant with the tube on the magnetic rack.
   ▲ Because of the differences among different magnetic racks, the adsorption time can be appropriately extended until the solution becomes totally clear.
2. Keep the 15 ml centrifuge tube stationary on the magnetic rack for 1 min, and discard all residual liquid by a pipette.
3. Remove the 15 ml centrifuge tube from the magnetic rack. Add **1 ml** Buffer WA to the tube and vortex the beads for 30 sec to resuspend the beads. Briefly centrifuge the 15 ml tube and transfer the mixture to a new 1.5 ml centrifuge tube. Load the 1.5 ml tube to the magnetic rack for 1 min or until the solution becomes clear (The 15 ml tube should be kept which can be reused in the next step to minimize product loss).
4. Keep the 1.5 ml centrifuge tube on the magnetic rack. Transfer the supernatant to the 15 ml tube from the previous step to rinse the residual magnetic beads, and transfer all the mixture to the 1.5 ml centrifuge tube again. Keep the 1.5 ml centrifuge tube on the magnetic rack for 2 min or until the solution is clear, and discard the supernatant completely with a pipette.

5. Remove the 1.5 ml centrifuge tube from the magnetic rack. Add **1 ml** Buffer WA to the tube to resuspend the beads by 30 sec vortexing. After brief centrifugation, load the 1.5 ml tube to the magnetic rack for 2 min or until the solution becomes clear, then discard the supernatant completely by pipetting (After discarding the supernatant, gently tap the magnetic rack against the bench to collect any residual liquid to the bottom of the tube, then remove it using a 200 µl pipette).
6. Remove the centrifuge tube from the magnetic rack. Add **1 ml** 80% ethanol to the tube to resuspend the beads by 30 sec vortexing. After brief centrifugation, load the 1.5 ml tube to the magnetic rack for 2 min or until the solution becomes clear, then discard the supernatant completely by pipetting.
7. Repeat step 6.
8. Keep the 1.5 ml centrifuge tube on the magnetic rack. Air dry the beads with the lid open at room temperature for 3 - 5 min. Gently tap the magnetic rack against the bench to collect any residual liquid to the bottom of the tube, then remove it using a 200 µl pipette.

Step 3: cfDNA elution (Elution Buffer is added according to Table 1)

1. Remove the 1.5 ml centrifuge tube from the magnetic rack. Add **30 µl** Elution Buffer to the tube and resuspend the beads by 5 min vortexing.
2. After brief centrifugation, load the 1.5 ml tube to the magnetic rack for 2 min or until the solution becomes clear, then transfer the cfDNA supernatant to a new centrifuge tube. The cfDNA product can be used immediately. The product can be kept at 2 - 8°C for short-term storage or -20°C for long-term storage.

08-3/Small Volume Sample (200 - 800 µl) cfDNA Extraction Procedure

Reagent preparation

1. Equilibrate the MagUltra Beads B at room temperature for 15 min, and mix thoroughly before use.
2. 80% ethanol (1 ml for each sample).
3. The adding volume of each component for different input volume is referred to Table 2:

Table 2. Reagent Preparation Worksheet for 200 - 800 µl Sample Input Volume

Components	Sample Volume				
	200 µl	400 µl	500 µl	600 µl	800 µl
Buffer ES	10 µl	20 µl	25 µl	30 µl	40 µl
Proteinase K	3 µl	6 µl	8 µl	12 µl	12 µl
Buffer L/B	300 µl	500 µl	630 µl	750 µl	1 ml
MagUltra Beads B	5 µl	10 µl	10 µl	10 µl	10 µl
Buffer WA			500 µl		
80% ethanol			2 × 500 µl		
Elution Buffer			15 - 50 µl		

Operation steps:

This procedure can refer to 08-2/Large Volume Sample (1 - 10 ml) cfDNA Extraction Procedure, and the following differences are listed below:

1. The adding volume of each component for different input volume is referred to Table 2.
2. Rinse with Buffer WA once.

08-4/High Concentration cfDNA Extraction Process

Reagent preparation

1. Equilibrate the MagUltra Beads B at room temperature for 15 min, and mix thoroughly before use.
2. 80% ethanol (3 ml for each sample).
3. For urine samples, isopropanol needs to be prepared additionally.
4. 0.1 × TAE (TAE Buffer is composed of Tris Base, Acetic Acid, and EDTA. 400 µl is required for each sample).
5. The adding volume of each component for different input volume is referred to Table 3 and Table 4:

Table 3. Reagent Preparation Worksheet for 4 - 10 ml Sample Input Volume

Components	Sample Volume			
	4 ml	6 ml	8 ml	10 ml
Buffer ES	200 µl	300 µl	400 µl	500 µl
Proteinase K	60 µl	90 µl	120 µl	150 µl
Buffer L/B	5 ml	7.5 ml	10 ml	12.5 ml
MagUltra Beads B	60 µl	90 µl	120 µl	150 µl
Isopropanol (For Urine Sample)	2 ml	3 ml	4 ml	5 ml
Buffer WA	2 × 1 ml			
80% ethanol	2 × 1 ml			
0.1 × TAE	400 µl			

Table 4. Reagent Preparation Worksheet for 400 µl Sample Input Volume

Components	Re-binding Sample Volume
	Elute from Table 3
Buffer L/B	500 µl
MagUltra Beads B	10 µl
Buffer WA	500 µl
80% ethanol	2 × 500 µl
Elution Buffer	30 µl

Operation steps

Step 1: Sample lysis and cfDNA binding

This procedure can refer to 08-2/Large Volume Sample (1 - 10 ml) cfDNA Extraction Procedure and the differences are listed below:

1. Isopropanol (urine samples only) is mixed with Buffer L/B, MagUltra Beads B and the sample. The adding volume of each component for different input volume is referred to Table 3.
2. Use 400 µl of 0.1 × TAE instead of Elution Buffer for elution.

Step 2: Re-binding

Use the product eluted with 400 µl of 0.1 × TAE as the sample. This procedure can refer to 08-2/Large Volume Sample (1 - 10 ml) cfDNA Extraction Procedure and the differences are listed below:

1. The adding volume of each component for different input volume is referred to Table 4.
2. Rinse with Buffer WA once.

08-5/Rapid Extraction Process

Reagent preparation

1. Equilibrate the MagUltra Beads B at room temperature for 15 min, and mix thoroughly before use.
2. 80% ethanol (2 ml for each sample).
3. The adding volume of each component for different input volume is referred to Table 5:

Table 5. Reagent Preparation Worksheet for 1 - 10 ml Sample Input Volume

Components	Sample Volume			
	1 ml	2 ml	4 ml	10 ml
Buffer L/B	1.25 ml	2.5 ml	5 ml	12.5 ml
MagUltra Beads B	15 µl	30 µl	60 µl	150 µl
Buffer WA	2 × 1 ml			
80% ethanol	2 × 1 ml			
Elution Buffer	20 µl	30 µl	60 µl	150 µl

Operating steps

This procedure is used for plasma separated from ordinary blood collection tubes only (For higher yield, the standard extraction protocol is recommended).

This procedure can refer to 08-2/Large Volume Sample (1 - 10 ml) cfDNA Extraction Procedure, and the differences are listed below:

1. Skip Buffer ES and Proteinase K treatment step in step one (sample lysis and cfDNA binding).

09/FAQ & Troubleshooting

Question	Reason	Solution
Question 1: Low product yields	The content of cfDNA in normal human plasma is between 1 - 100 ng/ml, and the exact content depends on the sample.	For low-level cfDNA samples, it is recommended to increase the sample input volume or use 08-4/ High concentration cfDNA extraction process. You can also refer to Question 4 for solutions.
Question 2: Low A260/280 and A260/230 raitos in cfDNA products	Due to cfDNA yield (comparing to other kinds of DNA extraction), the assessment of quantification and purity using absorbance spectrophotometry is usually inaccurate.	It is recommended to use Qubit to quantify nucleic acid content and protein residue content, and use Agilent 2100 or similar instruments to analyze the fragment distribution of the final products (the main peak of cfDNA distributes between 150 - 200 bp).
Question 3: Large fragment residue	Blood cell disruption may induce large fragment genomic DNA residual in products, which is related to blood quality and sample variability.	It is recommended to use freshly collected high-quality sample in cfDNA extraction.
Question 4: How to increase cfDNA yield	Nucleic acid co-precipitants can both enhance the binding of cfDNA to magnetic beads and reduce cfDNA loss caused by adsorption to consumables.	Nucleic acid co-precipitants can increase yield by 10% - 20%. For example, Carrier RNA may be used according to its instructions. It is recommended to customers who have verified that Carrier RNA does not affect downstream applications.

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