User Bulletin

ver. 18A

0.1 to 10 ml Serum or Plasma cfDNA Extraction Kit

Catalog No. CFD1001 25 preps

For Research Use Only

• Product description

0.1 to 10 ml Serum or Plasma cfDNA Extraction Kit provides a simple and reliable method for the rapid preparation of high-quality circulating cell-free DNA from serum or plasma. A combination of vacuum and spin method efficiently recover total cfDNA from 100 µl up to 10 ml of serum or plasma sample. The MM Vacuum-Spin column technology allows for ultra-pure DNA to be eluted in as little as 30 µl of Elution Buffer or water, the resulting DNA is suitable for all subsequent analyses and molecular manipulations such as qPCR, dPCR, Next-generation sequencing, and biomarkers discovery. Yield of 10–30 ng of cfDNA per ml of serum or plasma is typical. The majority of cfDNA fragments are approximately 150-170 bp.

Advantages

- Simple and robust high-quality cell-free DNA extraction from 100 µl to 10 ml of serum or plasma.
- MM Vacuum-Spin column technology enables elution of DNA in as little as 30 µl and ensures it is ready for all sensitive downstream applications such as qPCR, dPCR and Next-generation sequencing.
- Recover DNA as small as ≥ 50 bp, typical DNA recovery ranges from 1-100 ng/ml of serum or plasma.

Product Contents

Note - Integrity of kit components are guaranteed for up to one year from date of purchase.

Note –This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Catalog No.	**CFD1001**
Preps	25
FX Buffer	125 ml
PX Buffer	250 ml * 3
WN Buffer	50 ml * 2
WS Buffer	50 ml * 3
Elution Buffer	10 ml * 1
Proteinase K	10 mg * 10
MM Vacuum-Spin column	25
Collection Tube	50
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Store all buffers at room temperature, except proteinase K

All buffers need to be mixed well before use.

- **Required Equipment** – Vacuum manifold, water bath or heat block (55°C), microcentrifuge.

Please read the following notes before starting the procedures.

Important Notes

- Buffers in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn to protect from direct contact.
- All procedure should be done at room temperature (20-25 ℃).
- All centrifugation steps should be 10,000x g or 13,000 rpm in a microcentrifuge.
- This product is compatible with any conventional vacuum-based manifold with luer-fitting connector. The vacuum pump should be capable of producing up to 400 mm Hg pressure at the vacuum manifold.

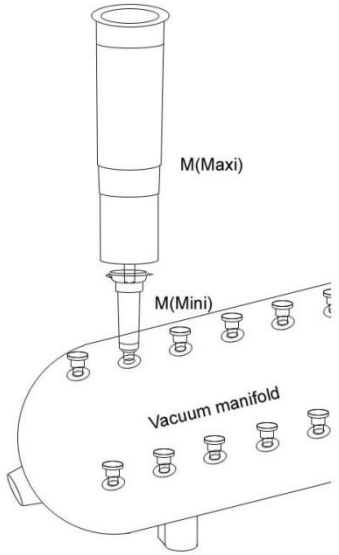
Reagent Preparation

- Prior to use, add 1 ml of autoclaved (DNase free) water to each Proteinase K (10 mg) tube. The final concentration of Proteinase K is 10 mg/ml, store at -20°C after mixing.
- Prior to use, add 200 ml 95-100% ethanol to the new WN Buffer, when first open.
- Prior to use, add 200 ml 95-100% ethanol to the new WS Buffer, when first open.

Protocol (Vaccum/Spin)

Drawing Note:

Insertion of a MM Vacuum-Spin column into a fitting-connector or a luer-fitting in up-right position onto a vacuum manifold.



1. Add 50 µl of FX (5X) Buffer to every 200 µl of serum or plasma and mix thoroughly. (Sample volumes other than 200 µl please see Reference Table for proper FX Buffer adding).

2. Add 20 µl of Proteinase K (20 mg/ml) to every 200 µl of serum or plasma of FX Buffer added, and mix thoroughly. (Sample volumes other than 200 µl please see Reference Table for proper Proteinase K adding). Incubate at 55 °C for 30 minutes for sample digestion.

3. Add 540 µl of PX Buffer to the proteinase K digested sample from step 2 and mix thoroughly. (Sample volumes other than 200 µl please see Reference Table for proper PX Buffer adding).

Reference Table

Serum/Plasma Sample Volume	100 µl	**200 µl**	1 ml	3 ml	5 ml	10 ml
FX (5X) Buffer	25 µl	**50 µl**	250 µl	750 µl	1.25 ml	2.5 ml
Proteinase K	10 µl	**20 µl**	100 µl	300 µl	500 µl	1 ml
Mix thoroughly and incubate at 55 °C for 30 minutes.						
Add PX Buffer	270 µl	**540 µl**	2.7 ml	8.1 ml	13.5 ml	27 ml
Insert a MM Vacuum-Spin column into a fitting-connector or a luer-fitting in up-right position onto a vacuum manifold, then gently pour PX mixed sample into a MM Vacuum-Spin column, and apply vacuum.						
Wash WN buffer (ethanol added)	500 µl	**1 ml**	4 ml	8 ml	12 ml	20 ml
Wash WS buffer (ethanol added)	1 ml	**2 ml**	8 ml	16 ml	20 ml	30 ml
Disconnect the MM Vacuum-Spin column from the vacuum manifold and break the MM Vacuum-Spin column connection by open-bull the cap of Mini column (bottom part), then transfer the mini column into a Collection Tube and centrifuge at full speed for 3 minutes in a microcentrifuge to ensure complete removal of the wash buffer.						
Place the washed Mini column into a new microcentrifuge tube (not provided), then add 30 µl to 200 µl DNA Elution Buffer or DNase free water, and centrifuge at full speed for 3 minutes in a microcentrifuge to elute cfDNA.						

4. Transfer (gently pour) the entire mixture (containing digested sample and PX Buffer) into MM Vacuum-Spin column set. Switch on the vacuum pump until the entire mixture is completely drawn through the column. Switch off the vacuum pump. Note: The capacity of MM column is about 30 ml, if more than 30 ml, apply several times to complete the sample application.

5. Wash the column by adding 1 ml of ethanol added WN Buffer. (Sample volumes other than 200 µl please see Reference Table for proper WN Buffer adding) to the column. Switch on the vacuum pump until the liquid has been completely drawn through the column.

6. Wash the column by adding 1 ml of ethanol added WS Buffer. (Sample volumes other than 200 µl please see Reference Table for proper WS Buffer adding) to the column. Switch on the vacuum pump until the liquid has been completely drawn through the column.

7. Disconnect the MM Vacuum-Spin column from the vacuum manifold and break the (Maxi-Mini column) MM Vacuum-Spin column connection by open-bull the cap of Mini column (bottom column), then transfer the Mini column into a Collection Tube and centrifuge at full speed for 3 minutes in a microcentrifuge to ensure complete removal of the wash buffer.

8. Transfer the Mini column into a 1.5 ml DNase/RNase-free tube (not provided). Add 30 µl to 200 µl DNA Elution Buffer or DNase free water directly to the column matrix. Incubate at room temperature for 3 minutes, then centrifuge at maximum speed for 3 minutes in a microcentrifuge. Eluted DNA can be used immediately for downstream applications or stored at -20°C.

Notes:

1. DNA Elution Buffer: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

2. The total yield may be improved by eluting the DNA with 60-70 ^{o}C DNA Elution Buffer; repeating elution may also help, by loading the first time elute back to membrane of the Mini column, incubate for 3 minutes at room temperature, and centrifuge again.

3. Generally, serum and plasma contain very low quantities of DNA, therefore Nanodrop is not recommended to quantify the DNA, other sensitive quantification methods, such as qPCR or dPCR may be used.

For technical assistance, please contact Viogene Research Technical Support via email to service@viogene.com.
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