Presto™ cfDNA/RNA Extraction Kit II

For research use only

Sample : 1-4 ml of serum or plasma

Yield : 1-100 ng of cfDNA / RNA per ml of serum or plasma

Format : cfDNA spin column combines with column extender

Operation procedure : vacuum or centrifugation

Operation time : within 40 minutes

Elution volume : 30-50 µl

Introduction

The Presto™ cfDNA/RNA Extraction Kit is designed for rapid isolation of high-quality circulating cell-free DNA/RNA from up to 4 ml of serum and plasma. The biological liquid sample is lysed with the Lysis Buffer containing chaotropic salts. The proteins in the sample lysate are then palleted with Precipitation Buffer and the cfDNA/RNA is bound with the CF column. The unique designed Column Extender allows the extraction procedure can be performed either using a vacuum manifold or a centrifuge with the swing bucket. The CF column is washed with Wash Buffer and cfDNA/RNA is eluted with RNase-free Water. The entire procedure can be completed within 40 minutes and the purified cfDNA/RNA is ready for use in all subsequent analyses and molecular manipulations such as qPCR, Next-Generation sequencing and DNA methylation analyses.

Quality Control

The quality of the Presto™ cfDNA/RNA Extraction Kit is tested on a lot-to-lot basis by isolating cfDNA/RNA from 1 ml of plasma. Following the purification process, the purified cfDNA/RNA integrity was assessed by qPCR.

Kit Contents

Product Name	CF002	CF025	CF050	CF100
CFL Buffer	4 ml	40 ml	80 ml	160 ml
CFP Buffer	1 ml	12 ml	25 ml	45 ml
Carrier RNA[1] (add RNase-free Water)	1 mg (1 ml)	1 mg (1 ml)	1 mg (1 ml)	1 mg (1 ml)
W1 Buffer	2 ml	30 ml	30 ml	50 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	12.5 ml (50 ml)	25 ml (100 ml)
RNase-free Water	6 ml	6 ml	6 ml	6 ml x2
CF Column	2 pcs	25 pcs	50 pcs	100 pcs
Column Extender	2 pcs	25 pcs	50 pcs	100 pcs
2 ml Collection Tube	2 pcs	25 pcs	50 pcs	100 pcs

[1]Carrier RNA is shipped at room temperature and should be stored at -20ºC once received the kit for extended periods. Add 1 ml of RNase-free Water to Carrier RNA then vortex to ensure it is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the Carrier RNA solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The Carrier RNA solution should be stored at -20ºC. Do not freeze and thaw the Carrier RNA solution more than 3 times.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements:

1.5 ml microcentrifuge tubes, 15 ml centrifuge tubes, 50 ml centrifuge tubes, absolute ethanol, isopropanol. Vacuum manifold for vacuum protocol and centrifuge with 50 ml centrifuge tube swing bucket for centrifuge protocol.

Step 1 Sample Lysis

- **CFL Buffer preparation:** Add **1 µl of Carrier RNA** to appropriate volume of **CFL Buffer** (see the table below) in a 15 ml centrifuge tube and vortex shortly to mix.

Sample volume	1 ml	2 ml	3 ml	4 ml
CFL Buffer	300 µl	600 µl	900 µl	1.2 ml
Carrier RNA	1 µl	1 µl	1 µl	1 µl

- Add appropriate volume of **serum** or **plasma** sample to the 15 ml centrifuge tube. Close the cap and mix by vortex for 30 seconds.

Note: In order to ensure efficient lysis, it is important that the sample and CFL Buffer are mixed thoroughly to yield a homogeneous solution.

- Incubate at room temperature for 3 minutes.
- Add **100 µl of CFP Buffer for each 1 ml of plasma or serum sample**. Close the cap and immediately mix vigorously by vortex for 30 seconds. Incubate on ice for 3 minutes.
- Centrifuge at 10,000 xg for 3 minutes to precipitate the protein pellet.

Note: If centrifuge at 10,000 xg is not available, centrifuge can be performed at 3,000 xg for 10 minutes.

- Transfer the clear supernatant (about 1 ml per ml of plasma or serum sample) to a clean 15 ml centrifuge tube and keep on ice.
- Add 1 volume of ice-cold isopropanol to the sample and mix well by vortex.

Step 2 cfDNA/RNA Binding

Centrifuge Protocol

- Connect the **CF column** with the **Column Extender**. Pressing the column lid down and slide the assembly into a clean 50 ml centrifuge tube.
- Transfer the entire sample mixture into the CF column assembly, close the centrifuge tube cap and centrifuge at 1,500 x g for 2 minutes. Discard the flow-through.
- Disconnect the CF column from the column extender and place the CF column in a **2 ml Collection Tube**.

Vacuum Protocol

- Connect the **CF column** with the **Column Extender** and place the CF column assembly onto a vacuum manifold.
- Transfer the entire sample mixture into the CF column assembly. Apply vacuum at 15 inches Hg until sample passes through the CF column assembly, switch off the vacuum.
- Disconnect the CF column from the column extender and place the CF column in a **2 ml Collection Tube**.

Connect the **CF column** with the **Column Extender**

Step 3 Wash	• Add **400 µl of W1 Buffer** to the **CF Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **CF Column** back in the 2 ml Collection Tube. • Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the **CF Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **CF Column** back in the 2 ml Collection Tube. • Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** to the **CF Column**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the **CF Column** back in the 2 ml Collection Tube. • Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.
Step 4 cfDNA/RNA Elution	• Transfer the dried **CF Column** to a new 1.5 ml microcentrifuge tube. • Add **30-50 µl of RNase-free Water**[1] into the **CENTER** of the column matrix. Let stand for at least 2 minutes to allow RNase-free Water to be completely absorbed. • Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified cfDNA/RNA. Eluted cfDNA/RNA can be used immediately for downstream applications or stored at ≤ -70°C. Note: [1]If a higher DNA/RNA concentration is required, use 30 µl of RNase-free Water then repeat the Elution step by adding the same 30 µl of RNase-free Water (which now contains the eluted DNA/RNA) to the center of the column matrix again. If maximum DNA/RNA yield is required, use 50 µl of RNase-free Water (DNA/RNA concentration will be diluted). Ensure that RNase-free Water is added into the center of the CF Column matrix and is completely absorbed.

Troubleshooting

Problem	Cause	Solution
Low nucleic acid yield	A. Primary blood tube contains an anticoagulant other than EDTA B. Wrong blood preservation condition C. Inappropriate buffer preparation	A. Anticoagulants other than EDTA may lead to accelerated DNA/RNA degradation compared to EDTA blood. Repeat the purification procedure with new samples. B. If plasma was prepared through an extended time after blood draw, blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid. In addition, if blood samples frozen and thawed more than once may lead to DNA/RNA degradation. C. Add appropriate volume of absolute ethanol (see the bottle label) to the Wash Buffer prior to use.
Eluted DNA/RNA does not perform well in downstream applications	A. Residual ethanol contamination B. Interference due to carrier RNA	A. Following the wash step, dry the CF Column with additional centrifugation at 16,000 x g for 3 minutes to remove residual ethanol. B. 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.