Presto™ cfDNA/RNA Extraction Kit

For research use only

Catalogue Numbers

CF025
CF050
CF100

Quantity

25 rxns
50 rxns
100 rxns

Geneaid

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Presto™ cfDNA/RNA Extraction Kit is designed for rapid isolation of high-quality circulating cell-free DNA/RNA from up to 5 ml of serum or plasma. The kit includes uniquely designed Column Extension Tubes which allow for increased sample volume and yield. Biological liquid samples are lysed using Proteinase K and a buffer containing chaotropic salt. The lysate is mixed with a binding buffer to facilitate cfDNA/RNA binding to the column membrane. The column is then washed and cfDNA/RNA is eluted with RNase-free Water. The entire procedure can be completed within 60 minutes and the purified cfDNA/RNA is ready for use in a variety of downstream applications such as qPCR, Next-Generation sequencing and DNA methylation analysis.

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 is assessed by qPCR.

Specifications

- Purify cfDNA/RNA within 60 minutes
- Sample: 1-5 ml of serum or plasma
- Format: cfDNA/RNA spin columns combined with column extension tubes using vacuum or centrifuge
- Yield: 1-100 ng of cfDNA/RNA per ml of serum or plasma
- Elution Volume: 30-50 µl
- Applications: qPCR, Next-Generation sequencing and DNA methylation analysis
- Storage: dry at room temperature (20~25ºC)

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask. Disposable/non-disposable glassware, plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.

Components

Catalogue Number	CF002	CF025	CF050	CF100
CF1 Buffer	10 ml	60 ml x 2	220 ml	220 ml x 2
CF2 Buffer[1]	6 ml x 2	150 ml	300 ml	300 ml x 2
(Add Isopropanol)	(4 ml x 2)	(100 ml)	(200 ml)	(200 ml x 2)
Proteinase K[2]	11 mg	135 mg	55 mg x 5	135 mg x 4
(Add RNase-free Water)	(1.1 ml)	(13.5 ml)	(5.5 ml x 5)	(13.5 ml x 4)
W1 Buffer	2 ml	30 ml	50 ml	50 ml
Wash Buffer[3]	1 ml	12.5 ml	12.5 ml	25 ml
(Add Ethanol)	(4 ml)	(50 ml)	(50 ml)	(100 ml)
RNase-free Water	6 ml	30 ml	6 ml, 30 ml	30 ml x 2
Carrier RNA[4]	1 mg	1 mg	1 mg	1 mg
(Add RNase-free Water)	(1 ml)	(1 ml)	(1 ml)	(1 ml)
Column Extension Tube	2 pcs	25 pcs	50 pcs	100 pcs
CF Column	2 pcs	25 pcs	50 pcs	100 pcs
2 ml Collection Tube	2 pcs	25 pcs	50 pcs	100 pcs

[1]Add Isopropanol (see bottle label for volume) to CF2 Buffer. Mix by shaking for a few seconds. Check the box on the bottle.
[2]Add RNase-free Water (see bottle label for volume) to Proteinase K then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the RNase-free Water and Proteinase K mixture should be stored at 4ºC.
[3]Add absolute ethanol (see 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.
[4]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.

Additional Requirements

1.5 ml microcentrifuge tubes, 50 ml centrifuge tubes, 60ºC water bath, absolute ethanol, isopropanol, vacuum manifold (vacuum protocol only) and a centrifuge with a 50 ml centrifuge tube swing bucket for (centrifuge protocol only).

cfDNA/RNA Purification Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. Sample Lysis

CF1 Buffer preparation

Combine **1 µl of Carrier RNA in RNase-free Water** with an appropriate volume of CF1 Buffer (see the table below) and vortex shortly to mix.

Sample volume	1 ml	2 ml	3 ml	4 ml	5 ml
CF1 Buffer	0.8 ml	1.6 ml	2.4 ml	3.2 ml	4 ml
Carrier RNA	1 µl	1 µl	1 µl	1 µl	1 µl

Add **Proteinase K, serum or plasma sample and CF1 Buffer containing Carrier RNA** to a 50 ml centrifuge tube in that order (see the table below). Close the cap and vortex for 30 seconds to mix.

1	**Proteinase K**	100 µl	200 µl	300 µl	400 µl	500 µl
2	**serum or plasma**	1 ml	2 ml	3 ml	4 ml	5 ml
3	**CF1 Buffer** containing carrier RNA	0.8 ml	1.6 ml	2.4 ml	3.2 ml	4 ml

NOTE: In order to ensure efficient lysis, it is important that the sample and CF1 Buffer are mixed thoroughly to yield a homogeneous solution.

Incubate in a 60ºC water bath for 30 minutes. Add **1 volume of CF2 Buffer (make sure isopropanol was added)** directly to **1 volume of sample mixture** (see the table below).

Sample volume	1 ml	2 ml	3 ml	4 ml	5 ml
CF2 Buffer	1.9 ml	3.8 ml	5.7 ml	7.6 ml	9.5 ml

Vortex the sample mixture for 10 seconds then incubate for 5 minutes on ice.

2. cfDNA/RNA Binding

Centrifuge Protocol

Connect the **CF Column to the Column Extension Tube**. Using your index finger, press the column lid down and slide the assembly into a clean 50 ml centrifuge tube. Transfer 10 ml of the sample mixture into the assembly and centrifuge at 1,500 x g for 2 minutes. Discard the flow-through and repeat until the entire sample mixture has passed through the column. Disconnect the column and place it in a 2 ml Collection Tube.

Vacuum Protocol

Connect the **CF Column to the Column Extension Tube** and connect the assembly to a vacuum manifold. Transfer the entire sample mixture into the assembly. Apply vacuum at 15 inches Hg until the sample passes through the column. Switch off the vacuum. Disconnect the column and place it in a 2 ml Collection Tube.

3. Wash

Add **400 µl of W1 Buffer** into the CF Column. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the column back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)**. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the 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.

4. Elution

Transfer the dry 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 ≤ -70ºC.

[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 Yield	A. Primary blood tube contains an anticoagulant other than EDTA. B. Incorrect blood preservation condition. C. Incorrect buffer preparation	A. Anticoagulants other than EDTA may lead to accelerated DNA/RNA degradation. Repeat the purification procedure with new samples. B. If plasma was prepared after an extended time following a blood draw, blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid. In addition, freezing and thawing blood more than once may lead to DNA/RNA degradation. C. Add appropriate volume of Isopropanol (see the bottle label) to CF2 Buffer and add appropriate volume of absolute ethanol (see the bottle label) to the Wash Buffer prior to use.
Eluted NA does not perform well in downstream applications	A. Residual ethanol contamination B. Carrier RNA interference	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 downstream enzymatic reactions, it may be necessary to reduce the amount of carrier RNA or omit it altogether.