FavorPrep™ Circulating Nucleic Acid Extraction HE Mini Kit

■ Kit Contents

Cat. No.	FACN0030 (4 Preps)	FACN0033 (50 Preps)	FACN0034 (100 Preps)
CL Lysis Buffer	15 ml	200 ml	400 ml
CB Binding Buffer (Concentrate) ▲	25 ml	135 ml x 2	270 ml x 2
Wash Buffer 1 (Concentrate) ■	0.48 ml x 2	12 ml	24 ml
Wash Buffer 2 (Concentrate) ●	3 ml	25 ml	50 ml
RNase-Free Water	0.5 ml	5 ml	8 ml
Proteinase K (Liquid)	1050 µl x 2	10.5 ml x 2	10.5 ml x 4
HE Column R	4 pcs	50 pcs	50 pcs x 2
HE Collection Tubes	4 pcs	50 pcs	100 pcs
Elution Tubes	4 pcs	50 pcs	100 pcs
Column Extenders	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of **CB Binding Buffer**, **Wash Buffer 1 and Wash Buffer 2**.			
Volume of 100% Isopropanol for CB Binding Buffer ▲	17 ml	90 ml	180 ml
Volume of 96~100% Ethanol for Wash Buffer 1 ■	0.72 ml	18 ml	36 ml
Volume of 96~100% Ethanol for Wash Buffer 2 ●	12 ml	100 ml	200 ml

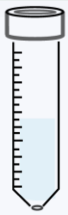
All kit components are shipped at room temperature and should be stored at room temperature between 15~25°C upon receipt.

■ Specification

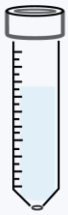
Format/Principle	Spin column (Silica matrix)
Binding Capacity	≤150 µg Nucleic acid/Column
Operation Time	<60 mins
Sample Size	1~4 ml cell-free fluid sample such as serums, plasma, or body fluids.
Elution Volume	30 µl

Procedure Overview

Sample

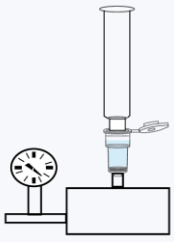


- Add 1~4 ml cell-free fluid sample into a tube.
- Add 0.1x sample volume of Proteinase K.
- Add 0.8x sample volume of CL Lysis Buffer.
- Mix thoroughly, incubate at 56˚C for 30 mins (vortex 2~3 times).



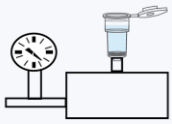
- Add 1.8x volume of CB Binding Buffer (isopropanol contained).
- Incubate the sample mixture on ice for 5 mins.

Vacuum
-650 mmHg



- Connect an HE Column R with a Column Extender.
- Transfer all the sample mixture into the HE Column R assembly.
- Pass the sample mixture through the HE Column R by vacuum pressure.

Vacuum
-650 mmHg



- Detach the Column Extender.
- Add 500 µl Wash Buffer 1 (ethanol contained).
- Add 900 µl Wash Buffer 2 (ethanol contained), repeat.

Centrifuge
18,000 xg, 2 mins

- Place the HE Column R to an HE Collection Tube.
- Dry the column membrane.

Centrifuge
18,000 xg, 2 mins

- Add 30 µl RNase-Free Water.
- Stand the column for 5 mins.
- Obtain purified nucleic acid.

■ Preparation Before Starting

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers. Make sure all materials are nuclease-free when executing extraction.
2. Additional materials: 96~100% ethanol, 100% isopropanol, and 15 ml or 50 ml centrifuge tube.
3. Additional equipment: vacuum source capable of -650 mmHg and vacuum manifold with an adaptor for column tip.
4. Add the indicated volume of isopropanol (100%) into the **CB Binding Buffer**, mix well; add the indicated volume of ethanol (96~100%) into the **Wash Buffer 1** and **Wash Buffer 2**, mix well. Store the buffer at room temperature.
5. Specimen storage and handling:
   a. To prevent the degradation of cell-free nucleic acids and the release of cellular nucleic acids, whole blood should be stored at 2~8˚C for no more than 6 hours. (e.g., in EDTA tube or specialized cfDNA collection tubes) Separate plasma or serum promptly.
   b. For short-term storage, the specimen can be stored at 2~8˚C for up to 24 hours after collection.
   c. For long-term storage, keep the specimen at −20°C or −80°C for up to 12 months for DNA, or at −80°C for up to 4 weeks for RNA.
   d. Make sure the cell-free fluid samples are clear. **Centrifuge the samples for 2 mins at 400 ×g if the debris are still visible.**
6. All centrifugation steps should be performed at **18,000 ×g**.

■ General Protocol: For 1~4 ml cell-free fluid

1. Add the indicated volume of **Proteinase K** and cell-free fluid sample into a 15 ml or 50 ml centrifuge tube (Refer to **Table 1/Step 1**), then mix thoroughly.
2. Add the indicated volume of **CL Lysis Buffer** to mixture then mix thoroughly (Refer to **Table 1/Step 2**).
3. Incubate the sample mixture at 56˚C for 30 mins. Vortex occasionally during incubation (2~3 times).
4. Add the indicated volume of **CB Binding Buffer** (isopropanol contained) to mixture then mix thoroughly (Refer to **Table 1/Step 4**).

Table 1. Quick Setup Guide

Step 1	**Sample Volume (ml)**	**1**	**2**	**3**	**4**
	Proteinase K (µl)	100	200	300	400
Step 2	CL Lysis Buffer (ml)	0.8	1.6	2.4	3.2
Step 3	Incubate the sample mixture at 56˚C for 30 mins.				
Step 4	CB Binding Buffer (isopropanol contained, ml)	1.8	3.6	5.4	7.2

5. Incubate the sample mixture on ice for 5 mins.
6. Connect an **HE Column R** with a **Column Extender** tightly then place the assembly on a vacuum manifold. Transfer the sample mixture carefully to the HE Column R assembly.
7. Pass all the sample mixture through the HE Column R by applying vacuum pressure until the column is empty. Detach the Column Extender.
8. Add 500 µl **Wash Buffer 1** (ethanol contained) to the HE Column R. Apply vacuum pressure until the column is empty.
9. Add 900 µl **Wash Buffer 2** (ethanol contained) to the HE Column R. Apply vacuum pressure until the column is empty.
10. Repeat **step 9**.
11. Place the HE Column R to an **HE Collection Tube**. Centrifuge the HE Column R for an additional 2 mins to dry the HE Column R.
12. Place the HE Column R to an **Elution Tube**, then add 30 µl of **RNase-Free Water** to the membrane center of the HE Column R. Stand the HE Column R for 5 mins.
    - **IMPORTANT!** For effective elution, make sure that RNase-Free Water is dispensed onto the membrane center and absorbed completely.
13. Centrifuge the HE Column R for 2 mins to elute nucleic acid.