User Manual

FavorPrep ™ Circulating Nucleic Acid Isolation Kit

-- For isolation of free-circulating nucleic acid from human plasma or serum

For Research Use Only

v MAR 21

Kit Contents:

Cat. No: (preps)	FACFK004 (4 preps)	FACFK050 (50 preps)
CL Lysis Buffer	20 ml	250 ml
CB Binding Buffer ● ***(concentrate)***	15 ml	165 ml x 2 bottles
CW1 Wash Buffer ■ ***(concentrate)***	*0.48 ml x 2*	20 ml
CW2 Wash Buffer ■ ***(concentrate)***	1.2 ml	15 ml
CE Elution Buffer	6 ml	30 ml x 2 bottles
Proteinase K ***(lyophilized)***	11 mg x 2 tubes	140 mg x 2 bottles
CF Column ***(nucleic acid binding column)***	4 pcs	50 pcs
Collection Tube	4 pcs	50 pcs
Elution Tube	4 pcs	50 pcs
Column Extender	4 pcs	50 pcs
User Manual	1	1

●, Add Isopropnal to concentrate CB Binding Buffer.
■, Add ethanol to concentrate CW1 Wash Buffer and CW2 Wash Buffer. ***See Working Buffer Preparation***.

Storage:

All buffers should be stored at room temperature (15-25 °C). **Lyophilized Proteinase K should be stored at 2~8 °C upon receipt.** And prepared Proteinase K solution should be stored at 2– 8°C.

Specification:

1. Principle: mini spin column (silica matrix)
2. Operation time: 30 ~ 60 minutes
3. Column applicability: vaccum and centrifugation
4. Minimum elution volume: 40 µl
5. Sample size: 1~ 5 ml human plasma or serum

Quality Control:

The quality of FavorPrep™ Circulating Nucleic Acid Isolation Kit is tested on a lot-to-lot basis according to ISO quality management system.

Important Note:

1. Make sure that the working environment is RNase-free.
2. Buffers provided by this kit containing irritants, wear gloves and lab coat for operation.
3. ***CAUTION:*** Buffers CL, CB and CW1 contain guanidinium salts which can form highly reactive compounds when combined with bleach. ***DO NOT add bleach or acidic solutions directly to the preparation waste.***
4. For handling the buffers safely, please read ***safety Information*** before starting the procedure.
5. This kit is suitable for the isolation of nuceic acid from fresh or frozen serum/ plasma prepared from blood collected on Heparin, EDTA or citrate.
6. Make sure the plasma or serum samples are clear. Centrifuge the samples for 2 minutes at 400 x g if the debris are still visible.
7. The vacuum source should be reached to - 650 mm Hg.
   - When using of vacuum to operate the nucleic acid extraction, ensure that the tip of the column is fit into the shape of manifold adaptor, and the vacuum pressure being capable to reach to - 650 mm Hg.
   - Units and values at same pressure (1 atm)

unit	value
atmosphere (atm)	1.000
millimeter of mercury (mmHg)	760.000
inches of mercury (inHg)	29.290
pascal (Pa)	101,325.000
kilopascal (KPa)	101.325
torr (torr)	760.000
pound per square inch (psi, 1bs/in²)	14.700

8. The centrifuge force should be performed at ~18,000 x g.
9. Add Isopropanol (96~100%) to CB Binding Buffer. And add ethanol (96-100%) to concentrate CW1 Wash Buffer and CW2 Wash Buffer before use. see ***Working Buffer Preparation.***

Materials and equipment provided by the user

1. Pipets and pipet tips.
2. Ethanol (96~100%)
3. Isopropanol (96~100%)
4. Crushed ice
5. Water bath or heating block for 1.5 ~ 2.0 ml microcentrifuge tubes and 50 ml centrifuge tubes at 60°C
6. Vacuum source capable of -650 mmHg.
7. Vacuum manifold with a adaptor for tip of the CF Column (nucleic acid binding column).
8. Microcentrifugator capable of ~ 18,000 x g for 1.5 or 2.0 ml microcentrifuge tubes.

Working Buffer Preparations:

1. Add CE Elution Buffer to lyophilized Proteinase K to make a 10 mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. Store the stock solution at 4 °C.
2. Add isopropanol to CB Binding Buffer and add ethanol to CW1 Wash Buffer and CW2 Wash Buffer when first use. Store the solution at room temperature (15~25 °C)

Cat No.: FACFK004 (4 preps)

Buffers	Preparations	volume after preparation
CL Lysis Buffer, 20 ml,15-25°C	----------	----------
CB Binding Buffer, (concentrate),15 ml	Add ***10 ml isopropanol*** (100 %), mix well, store at 15-25°C	25 ml
CW1 Wash Buffer, (concentrate),1.4 ml	Add ***0.72 ml ethanol*** (100%), mix well store at 15-25°C	1.2 ml
CW2 Wash Buffer, (concentrate),1.2 ml	Add ***4.8 ml ethanol*** (100%) and mix well store at 15-25°C	6 ml
CE Elution Buffer, 6 ml	----------	----------
Proteinase K (lyophilized), 11 mg x 2, 4~8 °C	Add ***1.1 ml*** of CE Elution Buffer to proteinase K tube. Dissolve well to make 10 mg/ml proteinase K solution and store at 4~8 °C	1.1 ml

Cat No.: FACFK050 (50 preps)

Buffers	Preparations	volume after preparation
CL Lysis Buffer, 250 ml,15-25°C	----------	----------
CB Binding Buffer, (concentrate), 165 ml x 2 bottles	Add ***110 ml isopropanol*** (100 %), mix well, store at 15-25°C	275 ml
CW1 Wash Buffer, (concentrate),20 ml	Add ***18 ml ethanol*** (100%), mix well store at 15-25°C	30 ml
CW2 Wash Buffer, (concentrate),15 ml	Add ***60 ml ethanol*** (100%) and mix well store at 15-25°C	75 ml
CE Elution Buffer, 30 ml x 2 bottles	----------	----------
Proteinase K (lyophilized), 140 mg x 2 bottles 4~8 °C	Add ***14 ml*** of CE Elution Buffer to proteinase K tube. Dissolve well to make 10 mg/ml proteinase K solution and store at 4~8 °C	14 ml

Safety Information:

CAUTION: CL Lysis Buffers, CB Binding Buffer and CW1 Wash Buffer contain guanidinium salts which can form highly reactive compounds when combined with bleach. ***DO NOT add bleach or acidic solutions directly to the preparation waste.***

Kit Component: **CL Lysis Buffer**, **CB Binding Buffer**

Hazard contents
Guanidinium thiocyanate
CAS-No. 593-84-0
EC-No. 209-812-1

GHS symbol  Warning

Hazard statement(s)

H302 + H312 + H332	Harmful if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.
P280	Wear protective gloves/ protective clothing / eye protection/ face protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Kit Component: **CW1 Wash Buffer**

Hazard contents
Guanidine hydrochloride, 20~50%, CAS-No. 50-01-1

GHS symbol  Warning

Hazard statement(s)

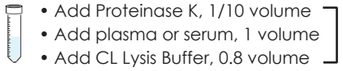
H302	Harmful if swallowed.
H319	Causes serious eye irritation.

Precautionary statement(s)

P264	Wash … thoroughly after handling.
P280	Wear protective gloves/ protective clothing/ eye protection/ fac protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.

Brief procedure:

STEP 1: Lyse



- Add Proteinase K, 1/10 volume
- Add plasma or serum, 1 volume
- Add CL Lysis Buffer, 0.8 volume

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- Plus-vortex , 10 sec

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- Incubate the sample mixture at 56~60 °C, 30 min

STEP 2: Bind (Vacuum)

- Add CB Binding Buffer (isopropanol added).

- Plus-vortex , 10 sec.

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- Incubate on ice 5 min

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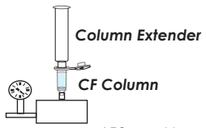
- Transfer sample mixture to the Column Extender



vacuum, -650 mmHg.

STEP 3: Wash (Vacuum)

- Add CW1 Wash Buffer (Ethanol added; 600 µl) x 1
- Add CW2 Wash Buffer (Ethanol added; 650 µl) x 2

vacuum, -650 mmHg.

STEP 4: Dry column's membrane

- Centrifuge, 18,000 x g, 3 min
- Incubate at 60 °C, 10 min

STEP 5: Elute

- Add CE Elution Buffer (≥40 µl)
- Stand for 3 min
- Centrifuge, 18,000 x g, 1 min

General Protocol: (For 1~ 5 ml plasma/serum sample)

Please Read Important Notes Before Starting Following Steps.

STEP 1: Lyse

1-1. Add 1/10 volume of Proteinase K Solution (10 mg/ml) to a 15 ml or 50 ml centrifuge tube.

For example:
Add 100 µl of Proteinase K for 1 ml of plasma/ serum sample, or add 400 µl of Proteinase K for 4 ml of plasma/serum sample.

1-2. Add 1 ~ 5 ml of plasma/serum sample. Mix well by plus-vortex for 10 sec.

1-3. Add 0.8 volume of CL Lysis Buffer. Mix well by vortexing.

For example:
Add 0.8 ml of CL Lysis Buffer for 1.0 ml of plasma/ serum sample, or add 3.2 ml of CL Lysis Buffer for 4.0 ml of plasma/ serum sample.

1-4. Incubate the sample mixture at 56~60 °C for 30 min. ***Vrotex occasionally during incubation. (2~3 times)***

STEP 2: Bind

2-1. Add 1.8 volume of CB Binding Buffer (Isopropanol added). ***Mix thoroughly by pulse-vortexing for 10 sec***.

For example:
Add 1.8 ml of CB Binding Buffer (Isopropanol added) for 1.0 ml of plasma/serum sample, or add 7.2 ml of CB Binding Buffer (Isopropanol added) for 4.0 ml of plasma/serum sample.

2-2. Incubate the sample mixture on ice for 5 min. Connect a CF Column with a Column Extender then combine it with a vacuum manifold.

2-3. (Vacuum) Pass all the sample mixture through the CF Column by applying vacuum at -650 mm Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.

STEP 3: Wash

3-1. Depart the Column Extender.

3-2. (Vacuum) Add 500 µl of **CW1 Wash Buffer** to the CF Column. Pass all the CW1 Wash Buffer through the CF Column by applying vacuum at -650 mm Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.

3-3. (Vacuum) Add 650 µl of **CW2 Wash Buffer** to the CF Column. Pass all the CW2 Wash Buffer through the CF Column by applying vacuum at -650 mm Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.

3-4. Repeat step 3-3 for one more washing.

STEP 4: Dry the column's membrane

4-1. Cap the CF Column and place the column to a Collection Tube. Centrifuge the combined column at 18,000 x g for 3 min.

4-2. Transfer the CF Column to a Elution Tube. Open the cap and incubate the combined column at 60 °C for 10 min.

STEP 5: Elute

5-1. Add ≥ 40 µl of CE Elution Buffer to the membrane of the CF Column. Stand the CF Column for 3 min at room temperature.

5-2. Centrifuge the combined column at 18,000 x g for 1 min to elute the nucleic acid.

Product category of Favorgen:

For more information please visit Favorgen web site *www.favorgen.com*

Nucleic Acid Extraction - spin column (silica membrane)
- Viral DNA/ RNA Kit
- Viral Nucleic Acid Extraction Kit II
- Viral RNA/ DNA Vacuum Kit
- Circulating Nuleic Acid Isolation Kit

RNA Extraction - spin column (silica membrane)
- Blood/Cultured Cell Total RNA ***Mini/ Maxi*** Kit
- Soil RNA Isolation Mini Kit
- Tissue Total RNA ***Mini/ Maxi*** Kit
- Plant Total RNA ***Mini/ Maxi*** Kit
- After Tri-Reagent RNA Clean-Up Kit

96-Well high throughput DNA/ RNA extraction (silica membrane)
- 96-well Gel/ PCR purification kit
- 96-well PCR Clean-Up Kit
- 96-Well Total RNA Kit
- 96 well Viral DNA/RNA extraction kit
- 96-Well Genomic DNA Extraction Kit
- 96-Well Plasmid Kitsin)

DNA Clean-Up - spin column (silica membrane)
- PCR Clean-UP Kit/ • GEL Purification Kit/ • GEL/PCR Purification Kit
- ***MicroElute*** GEL/PCR Purification Kit

DNA Extraction - spin column (silica membrane)
- Blood / Cultured Cell Genomic DNA Extraction ***Mini /Midi/ Maxi*** Kit
- Plant Genomic DNA Extraction ***Mini/ Maxi*** Kit
- Food DNA Extraction Kit
- Milk Bacterial DNA Extraction Kit
- Tissue Genomic DNA Extraction Mini Kit
- FFPE Tissue DNA Extraction ***MicroElute*** Kit
- Fungi/ Yeast Genomic DNA Extraction Mini Kit
- Soil DNA Isolation Mini Kit
- Stool DNA Isolation Mini Kit

Extraction Reagent
- Tri-RNA Reagent - (Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction)

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