User Manual

FavorPrep™ Viral DNA/RNA Kit

- For isolation of viral DNA/RNA from cell-free fluid such as, serum, plasma, body fluid and cell culture supernatant, and from transport medium of swabs

For Research Use Only

Kit Contents:

Cat. No:	FAVNK 000-1 (4 preps)	FAVNK 001 (50 preps)
VNE Buffer ■	1.8 ml x 2	35 ml
Carrier RNA ■	0.04 mg	0.4 mg
Wash Buffer 1 (concentrate) □	0.48 ml x 2	12 ml
Wash Buffer 2 (concentrate) □	1.5 ml	20 ml
RNase-free Water	0.5 ml	6 ml
VNE Column	4 pcs	50 pcs
Collection Tube	8 pcs	100 pcs
Elution Tube	4 pcs	50 pcs
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■ ***See Working Buffer Preparation***.
□ Adding Ethanol to the concentrate Wash Buffer. ***see Working Buffer Preparation***.

Cat. No:	FAVNK 001-1 (100 preps)	FAVNK 001-2 (300 preps)
VNE Buffer ■	70 ml	200 ml
Carrier RNA ■	0.8 mg	2.2 mg
Wash Buffer 1 (concentrate) □	24 ml	72 ml
Wash Buffer 2 (concentrate) □	20 ml x 2	50 ml x 2
RNase-free Water	12 ml	20 ml
VNE Column	100 pcs	300 pcs
Collection Tube	200 pcs	600 pcs
Elution Tube	100 pcs	300 pcs
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■ ***See Working Buffer Preparation***.
□ Adding Ethanol to the concentrate Wash Buffer. ***see Working Buffer Preparation***.

Storage:

1. Kit components (except Carrier RNA) should be stored at room temperatures between 15~25°C.
2. Carrier RNA should be stored at -20°C upon receipt.
3. VNE Buffer should be stored at 4°C after adding Carrier RNA.

Quality Control:

The quality of FavorPrep™ Viral DNA/RNA Kit is tested on a lot-to-lot basis according to ISO quality management system.

Description:

FavorPrep™ Viral DNA/RNA Kit is an excellent tool for extraction of highly pure viral nucleic acid from viral cell-free specimens such as, serum, plasma, body fluids and cell-cultured supernatant, via transport medium of swabs. It is exclusively intended for this use by trained professionals.

Protocol overview:

The extraction method is silica-based with a chaotropic salt technology presence. The procedure involves lysis of virus for optimization of a binding condition that promotes the efficient penetration of the viral nucleic acid into the silica membrane. Carefully carried out centrifugation/vacuum protocols, plus Wash Buffers 1&2 guarantee contaminants including salts, metabolites, nucleases, and other components of body fluids are removed. Finally, highly pure Viral RNA is eluted from the silica membrane.

The provided Carrier RNA in conjunction with the unique lysis buffer enhance the process of binding nucleic acid to the silica membrane. This produces a substrate cleaved by the nuclease contained in the sample mixture; thus, elevating the integrity and recovery of viral nucleic acid. When compared with other harmful and time-consuming methods, such as phenol/chloroform extraction and ethanol precipitation, the FavorPrep™ Viral DNA /RNA Kit makes the extraction of high-purity viral nucleic acid reliable and efficient by shortening the handling time by 20 minutes per preparation.

Specification:

Format/Principle: spin column/silica membrane/chaotropic salt
Sample size: 140 µl cell-free fluid such as serum, plasma, body fluid and cell cultured supernatant
Operation time: <20 mins
Recovery rate: 80~90%
Length of recovery nucleic acid: >200 bp
Column Binding capacity: ≤60 µg/column
Elution volume: 30~60 µl
Column applicability: centrifugation and vacuum

Materials and equipment provided by the user

For centrifuge processing:

1. A microcentrifugator with a rotor for1.5 or 2.0 ml microcentrifuge tubes.
   • The centrifuge force should reach ~18,000 xg.
2. Sterile pipets, pipet tips and centrifuge tubes (1.5 ml, 2.0 ml).
3. 100 % ethanol
4. Vortexer
5. Tube heater set to 60°C and 70°C for 1.5 or 2.0 ml microcentrifuge tubes

For vacuum processing:

1. A microcentrifugator with a rotor for 1.5~2.0 ml micro-centrifuge tubes.
   • The centrifuge force should reach ~18,000 xg.
2. A vacuum manifold containing adaptors for VNE Column.
   • The vacuum source should reach -6 inches Hg.
   • Make ensure that the the manifold adaptor is fitable to the VNE column tip.
   • 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

3. Sterile pipets, pipet tips and centrifuge tubes (1.5 ml, 2.0 ml)
4. Microcentrifuge capable of speed at ~18,000 rpm
5. 100% ethanol
6. Vortexer
7. Tube heater set to 60°C and 70°C for 1.5 or 2.0 ml

Working Buffer Preparations:

■ **Preparation of VNE-Carrier RNA Buffer**
Add 1 ml of VNE Buffer to the tube containing lyophilized Carrier RNA. Mix well by vortexing and transfer the mixture to the VNE Buffer when first open. Store the VNE-Carrier RNA Buffer at 4°C.

□. **Preparation of Wash Buffer 1 and Wash Buffer 2**
Add required ethanol (96~100%) as the table below indicated. Store the Wash Buffer 1 & 2 (ethanol added) at 15~25°C.

Cat. No:	FAVNK000-1 (4 preps)	FAVNK001 (50 preps)	FAVNK001-1 (100 preps)	FAVNK 001-2 (300 preps)
Ethanol for ***Wash Buffer 1***	0.72 ml	18 ml	36 ml	108 ml
Ethanol for ***Wash Buffer 2***	6 ml	80 ml	80 ml	200 ml

Safety Information:

CAUTION: VNE Buffers and Wash Buffer 1 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: **VNE 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: **Wash Buffer 1**

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.

Important notes:

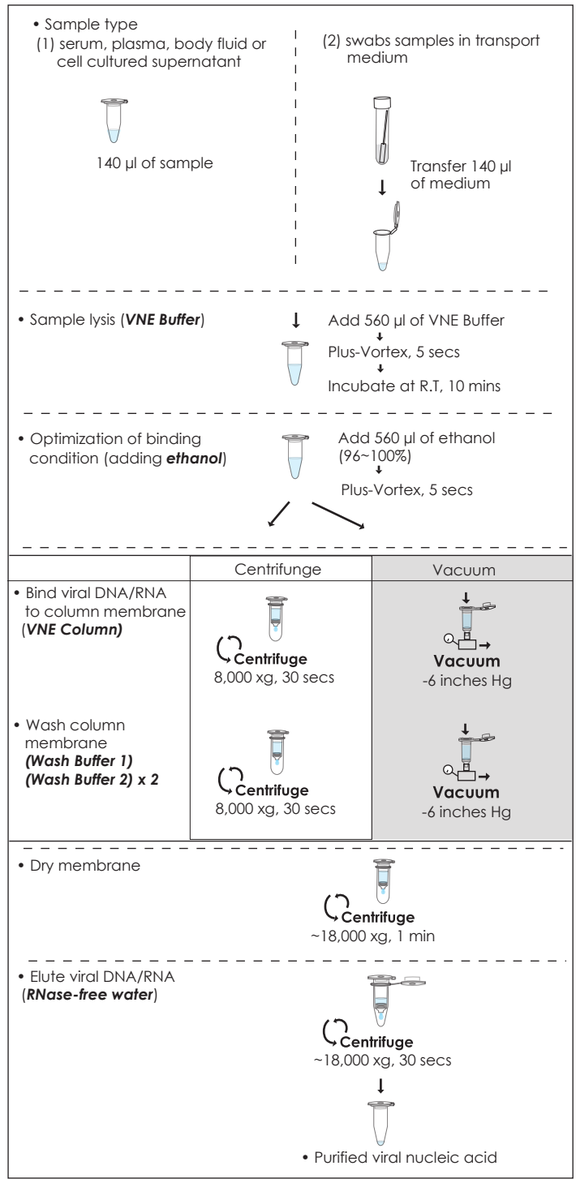
1. Notes for sample preparation:
- Make sure everything is RNase-free when handling this system.
- Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
- Do not thaw the frozen plasma or serum samples more than once.
- Centrifuge the plasma or serum samples at 6,000 xg for 3 mins If precipitates are visible. Then transfer the cleared supernatant to a new vial and processed immediately.

2. Notes for Buffers:
- Add Carrier RNA to the VNE Buffer when first open. Store the VNE-Carrier RNA Buffer at 4°C. see ***Working Buffer Preparation.***
- Add required ethanol (96~100%) to Wash Buffer 1 and Wash Buffer 2 before use. see ***Working Buffer Preparation.***
- For handling the buffers safely please read ***safety Information*** before starting the procedure.

3. Notes for centrifugation and vacuuming:
- Ensure that centrifugation speed corresponds to the instruction of individual steps.
- When using the vacuum to proceed "DNA/RNA to column membrane" and "Wash column membrane", ensure that the tip of the column fits nicely into the manifold adaptor, and vacuum pressure has a capacity of achieving -6 inches 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

Brief procedure:



Centrifuge Protocol:

Please Read Important Notes Before Starting Following Steps.

• Sample type

A. Cell-free fluid such as Serum, plasma, body fluids and cell cultured supernatant

1-A1. Briefly spin the tube to descend the drops attached on the tube wall. **Note!** Cntrifuge the sample at 7,000 xg for 3 mins If the precipitates are visible.

1-A2. Transfer 140 µl of the fluid sample (cleared supernatant) to a microcentrifuge tube (not provided).

B. Medium of transport swabs

1-B1. Briefly vortex the swabs transport tube then briefly spin the tube to descend the drops attached on the tube wall.

1-B2. Transfer 140 µl of the medium to a microcentrifuge tube (not provided).

• Sample lysis

2. Add 560 µl of VNE-Carrier RNA Buffer (Carrier RNA added, **see Working Buffer Preparation**). Mix well by vortexing and incubate for 10 mins at room temperature.

• Optimization of binding condition

3. Add 560 µl of ethanol (96~100%) to the sample mixture and mix well by plus-vortexing.

• Bind viral DNA/RNA to column membrane (centrifuge)

4. Combine a VNE column with a Collection Tube (provided). Transfer up to 700 µl of sample mixture (ethanol added) to the VNE Column. Centrifuge at 8,000 xg for 1 min then discard the flow-through. Combine the VNE Column with the used Collection Tube.
5. Transfer the rest of sample mixture (ethanol added) to the VNE Column and centrifuge at 8,000 xg for 1 min. Discard the flow-through and the Collection Tube. Combine the VNE Column with a new Collection Tube (provided).

• Wash column membrane (centrifugation)

6. Add 500 µl of Wash Buffer 1 (ethanol added) to the VNE Column. Centrifuge at 8,000 xg for 1 min then discard the flow -through. Combine the VNE Column with the used Collection Tube.
   -- Make sure that ethanol (96~100%) has been added into Wash Buffer 1 when first open.
7. Add 650 µl of Wash Buffer 2 (ethanol added) to the VNE Column. Centrifuge at 8,000 xg for 1 min then discard the flow-through. Combine the VNE Column with the used Collection Tube.
   -Make sure that ethanol (96~100%) has been added into Wash Buffer 2 when first open.
8. Repeat step 7.

• Dry membrane

9. Centrifuge at full speed (~18,000 xg) for 1 min to dry the VNE Column. Discard the flow-through and the Collection Tube.
   -Important step! This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.

• Elute Viral RNA

10. Combine the VNE Column with a Elution Tube (provided). Add 30~60 µl of RNase-free Water to the membrane center of the VNE Column. Stand VNE Column for 1 min.
    -Important step! For effective elution, make sure that the RNase-free Water is dispensed onto the membrane center and is absorbed completely.
11. Centrifuge at full speed (~18,000 xg) for 1 min to elute the viral DNA/RNA. Store the viral DNA/RNA at -70°C.

Vacuum Protocol:

Please Read Important Notes Before Starting Following Steps.

• Sample type

A. Cell-free fluid such as Serum, plasma, body fluids and cell cultured supernatant

1-A1. Briefly spin the tube to descend the drops attached on the tube wall. **Note!** Cntrifuge the sample at 7,000 xg for 3 mins If the precipitates are visible.

1-A2. Transfer 140 µl of the fluid sample (cleared supernatant) to a microcentrifuge tube (not provided).

B . Medium of transport swabs

1-B1. Briefly vortex the swabs transport tube then briefly spin the tube to descend the drops attached on the tube wall.

1-B2. Transfer 140 µl of the medium to a microcentrifuge tube (not provided).

• Sample lysis

2. Add 560 µl of VNE-Carrier RNA Buffer (Carrier RNA added, **see Working Buffer Preparation**). Mix well by vortexing and incubate for 10 mins at room temperature.

• Optimization of binding condition

3. Add 560 µl of ethanol (96~100%) to the sample mixture and mix well by plus-vortexing.

• Bind viral DNA/RNA to column membrane (vacuum)

4. Combine the tip of a VNE Column with the adaptor of the vacuum manifold. Retain the Collection Tube for be used on step 9. Transfer up to 700 µl of sample mixture (ethanol added) to the VNE Column and apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
5. Transfer the rest of the sample mixture (ethanol added) to the VNE Column and apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.

• Wash column membrane (vacuum)

6. Add 500 µl of Wash Buffer 1 (ethanol added) to the VNE Column. Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
7. Add 650 µl of Wash Buffer 2 (ethanol added) to the VNE Column Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
8. Repeat step 7.

• Dry membrane

9. Remove the VNE Column from manifold and return the VNE Column back to the Collection Tube. Centrifuge at full speed (~18,000 xg) for 1 min to dry the VNE Column. Discard the flow-through and the Collection Tube.
   -Important step! This step will avoid the residual liquid to inhibit the subsequent enzymatic reactions.

• Elute Viral RNA

10. Combine the VNE Column with a Elution Tube (provided). Add 30~60 µl of RNase-free Water to the membrane center of the VNE Column. Stand VNE Column for 1 min.
    -Important step! For effective elution, make sure that the RNase-free Water is dispensed onto the membrane center and is absorbed completely.
11. Centrifuge at full speed (~18,000 xg) for 1 min to elute the viral DNA/RNA. Store the viral DNA/RNA at -70°C.

Troubleshooting

• Low yield

■ Carrier RNA not add to VNE Buffer or VNE-Carrier Buffer not store well
- □ Add 1 ml of VNE Buffer to Carrier RNA. Mix well and transfer the to the VNE Buffer and store the VNE-Carrier RNA Buffer at 4°C.

■ Sample not store well or thaw repeatly
- □ Store samples at –80°C for long-term storage. Frozen samples do not be thawed more than once.

■ RNA Degradation
- □ Harvested samples not immediately stabilized.

■ Insufficient mixing with VNE-Carrier RNA Buffer
- □ Mix the sample mixture by plus-vortexing

■ Insufficient lysis of protein
- □ Incubate the sample mixture at room temperature for 10 mins after adding VNE-Carrier RNA Buffer.

■ Improper RNA binding condition
- □ No ethanol added to the lysate (step 3) or incorrect percentage of ethanol be used.

■ Incorrect RNA elution
- □ Ensure that RNase free water was added at the center of the VNE column membrane and absorbed by the membrane .

■ Incorrect preparation of Wash Buffer 1&2
- □ Ensure that the correct volume of ethanol (96~100%) was added to Wash Buffer 1&2 when first use.

• Eluted RNA does not perform well

■ Residual ethanol contamination
- □ Ensure that VNE Column has done centrifugation for an additional 1 min at speed ~18,000 xg (step 9) after washing step.

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
- 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 Kit

Plasmid Extraction
- ***Mini/Midi/Maxi*** plasmid kit - spin column (silica membrane)
- ***Midi/Maxi*** plasmid kit - gravity flow column (anion-exchange resin)
- Endotoxin Free ***Midi/Maxi*** plasmid kit - gravity flow column (anion-exchange resin)

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)

Telephone: +886-8-762-1829 (Taiwan)
Fax: +886-8-762-0791(Taiwan)
web site: *www.favorgen.com*
E-Mail: order@favorgen.com
technical@favorgen.com