User Manual

FavorPrep™ Viral Nucleic Acid Extraction 96-Well Kit

- For 96-well high-throughput extraction of viral nucleic acid from cell free samples such as serum, plasma, body fluids and the supernatants of cell cultures

Kit contents:

Cat. No.: (Q'ty)	FAVN207A (1 plate)	FAVN207B (2 plates)	FAVN207C (4 plates)
VNE Buffer	60 ml	120 ml	120 ml × 2
AD Buffer ▲	5 ml	10 ml	10 ml × 2
Wash Buffer 1 ■ (concentrate)	55 ml	110 ml	110 ml × 2
Wash Buffer 2 □ (concentrate)	25 ml	50 ml	50 ml × 2
RNase-free water	15 ml	30 ml	30 ml × 2
Filter Plates (96-Well DNA/RNA Binding plate)	1 plate	2 plates	4 plates
Collection Plates (96-Well 2 ml Plate)	3 plates	6 plates	12 plates
Elution Plates (96-Well PCR plate)	1 plate	2 plates	4 plates
Adhesive Film	2 pcs	4 pcs	8 pcs

▲, ■, □ : Add ethanol (96~100%) to AD Buffer, Wash Buffer 1 and Wash Buffer 2 when first use. See ***Working Buffer Preparation***.

Storage:

All components of FavorPrep™ Viral Nucleic Acid Extraction 96-Well Kit should be stored at room temperature (15~25°C).

Quality control

The quality of FavorPrep™ Viral Nucleic Acid Extraction 96-Well Kit is tested on a lot-to-lot basis according to ISO quality management system. The purified nucleic acid is checked by real-time PCR and capillary electrophoresis.

Product description:

FavorPrep™ Viral Nucleic Acid Extraction 96-Well Kit is an excellent tool for 96-well high-through put extraction of high pure viral nucleic acid from viral cell free specimen such as, serum, plasma, body fluid and cell cultured supernatant, and from transport medium of swabs. The extraction method is based on the silica membrane/chaotropic salt technology, and the procedure involves lysis of virus, optimization of binding condition being able to make the viral nucleic acid efficiently to silica membrane, washing silica membranes to remove contaminations including salts, metabolites, nucleases and other components of body fluid, finally elution of the viral nucleic acid from the silica membrane. Compare with other harmful and time-consuming method, such as phenol/chloroform extraction and ethanol precipitation, FavorPrep™ Viral Nucleic Acid Extraction 96-Well Kit makes extraction of high-purity viral nucleic acid reliable, and that shortens the handling time less than 60 mins for a 96-preparations.

Specification:

Principle: Filter Plate (glass fiber membrane)
Sample size: 200 µl of serum, plasma, body fluids and the supernatant of cell cultures
Plate applicability: vacuum or centrifugation
Operation time: within 1 hour/96 preparations
RNA Binding capacity: up to 60 µg/well
Elution volume: 50~75 µl

Additional materials required

For All Protocol:
- Pipets and pipet tips, sterile
- 96~100% nuclease-free ethanol
- 96-Well PCR Rack

For vacuum processing:
- A centrifuge capable of reaching a minimum speed of 5,600~6,000 xg with a microplate swinging-bucket rotor (capable of accommodating an 8.0 cm plate stack).
- A vacuun manifold for 96-well plate and a vaccum source reached to -12 inches Hg are required.

For centrifuge processing:
- A centrifuge capable of reaching a minimum speed of 5,600~6,000 xg with a microplate swinging-bucket rotor (capable of accommodating an 8.0 cm plate stack).

Working Buffer Preparations:

Preparation of AD Buffer ▲, Wash Buffer 1 ■ and Wash Buffer 2 □
Add required ethanol (96~100%) to AD Buffer, Wash Buffer 1 and Wash Buffer 2 as the table indicated. Store the ethanol added AD Buffer, Wash Buffer 1 and Wash Buffer 2 at 15~25°C.

Cat. No.:	FAVN207A (1 plate)	FAVN207B (2 plates)	FAVN207C (4 plates)
▲ Ethanol volume for **AD Buffer**	40 ml	80 ml	80 ml
■ Ethanol volume for **Wash Buffer 1**	10 ml	20 ml	20 ml
□ Ethanol volume for **Wash Buffer 2**	100 ml	200 ml	200 ml

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 required ethanol (96~100%) to AD Buffer, 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 centrifuging and vacuum:
- Ensure that centrifugation speed is according to instruction of individual step.
- When using of vacuum to proceed "DNA/RNA Binding" and "Wash the Filter Plate", ensure the vacuum pressure being capable to reach to -12 inches Hg.

4. **Notes for sealed plate**
- Ensure plates are tightly sealed with **Adhesive Film** to prevent sample loss or cross-well contamination. Never reuse adhesive film.

Brief procedure:

• STEP 1. Sample preparation and lysis

Collect samples in a Collection Plate (firet collection plate) →  Add VNE Buffer →  Mix by pipetting → Stand at room temperature for 10 min

• STEP 2. Adjust binding condition:

 Add AD Buffer →  Mix by pipetting

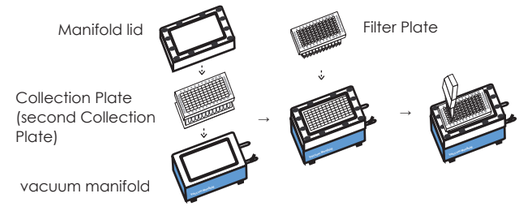
↙ ↘

Vacuum protocol or Centrifuge protocol

• STEP 3. Bind DNA/RNA to Filter Plate:

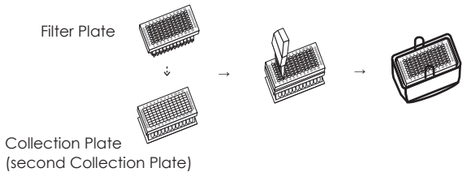
Vacuum processing

- Fix Plates to manifold.
- Transfer the sample mixture to Filter plate.
- Apply -12 inches Hg vacuum until the wells have emptied.



Centrifuge processing

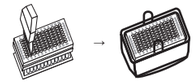
- Combind the plates.
- Transfer the sample mixture to Filter plate.
- Centrifuge at 5,600~6,000 xg for 2 mins.



• STEP 4. Wash the Filter Plate with Wash Buffer 1

- Add Wash Buffer 1.
- Apply vacuum at -12 inches Hg.

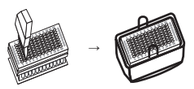
- Add Wash Buffer 1.
- Centrifuge at 5,600~6,000 xg for 2 mins.



• STEP 5 & 6. Wash the Filter Plate with Wash Buffer 2

- STEP 5 : Add Wash Buffer 2. Apply vacuum at -12 inches Hg.
- STEP 6: Add Wash Buffer 2 Apply vacuum at -12 inches Hg for **10 mins**.

- STEP 5: Add Wash Buffer 2. Centrifuge at 5,600~6,000 xg for 2 mins
- STEP 6: Add Wash Buffer 2. Centrifuge at 5,600~6,000 xg for **15 mins**



• STEP 7. Dry the membranes of the Filter Plate:

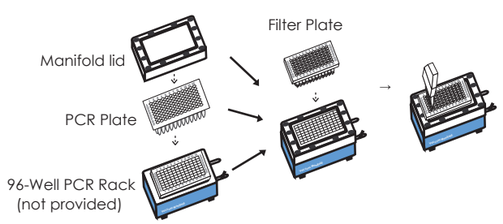
- Tap the Filter Plate tips on paper towel
- Return the Filter Plate and the Collection Plate to the manifold.
- Apply maximum vacuum for an additional 10 mins.

- Stand the Filter plate on a clean paper towel at room temperature for 10 mins.

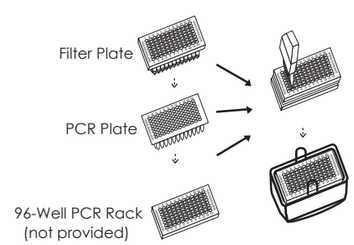
• STEP 8. DNA/RNA Elution:

- Add RNase-Free Water to the Filter Plate. Stand for 3 min.
- Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- Open the manifold valve to apply vacuum to elute viral DNA/RNA.

Alternative : If the consistent volume of elutes are recommended, use centrifuge protocol to process this elution step. (Page 4, STEP 8).



- Add RNase-Free Water to the Filter Plate. Stand for 3 mins.
- Centrifuge to elute viral DNA/RNA.



Safety Information:

1. VNE Buffer and Wash Buffer 1 provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. ***CAUTION:*** VNE Buffers and Wash Buffer 1 contains guanidinium salts which can form highly reactive compounds when c ombined 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  Danger

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.

Protocol: Vacuum processing

Please read Important Notes and Additional Materials Required before starting the following steps.

Required hardware
Vacuun manifold for 96-well plate and vaccum source reached to -12 inches Hg

Alternative: If using centrifugation for Elution Step (STEP 8), a centrifuge equiment is required, capable of 5,600~6,000 xg, with a swing-bucket rotor and the adaptor for 96-well plate.

STEP 1. Sample preparation and lysis
- Transfer 200 µl of sample to each well of a Collection Plate (provided, 96-well 2 ml plate; first collection plate).
  -If prepared samples are less than 200 µl, adjust sample volume to 200 µl with PBS (not provided).
- Add 400 µl of VNE Buffer to each well and mix completely by pipetting.
- Incubate at room temperature for 10 mins.

STEP 2. Adjust binding condition
- Add 300 µl of AD Buffer (ethanol added) to each well and mix completely by pipetting.

STEP 3. DNA/RNA Binding
- Fix a clean Collection Plate (provided, second collection plate) on the rack of vacuum manifold and cover the manifold lid. Place a Filter Plate (provided, 96-Well nucleic acid binding plate) on top of the Collection Plate (second collection plate).
- Transfer the sample mixture to the Filter Plate and discard the Collection Plate (first collection plate).
- Apply vacuum at -12 inches Hg until the wells have emptied.
- Discard the Collection Plate (second).
- Place the Filter Plate and a clean Collection Plate (provided, third collection plate) to the manifold.

STEP 4. Wash the Filter Plate with Wash Buffer 1
- Add 500 µl of Wash Buffer 1 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at -12 inches Hg until the wells have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.

STEP 5. Wash the Filter Plate with Wash Buffer 2
- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at -12 inches Hg until the wells have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.

STEP 6. Wash the Filter Plate again with Wash Buffer 2
- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at -12 inches Hg for 10 mins.
- Discard the flow-through and return the Collection Plate to the manifold.

STEP 7. Dry the membranes of Filter Plate
- Gently tap the tips of the Filter Plate on a clean paper towel to remove residual liquid.
- Return the Filter Plate to the Collection Plate fixed in the manifold.
- Apply vacuum for an addition 10 mins.
- Discard the Collection Plate (third).

STEP 8. DNA/RNA Elution
- Place an Elution Plate (provided, 96-Well PCR Plate) on the 96-Well PCR Rack (not provided) and fix plate onto manifold. Cover the manifold lid and place the Filter Plate on the Elution Plate (top: Filter Plate; middle: 96-Well PCR Plate; bottom: 96-Well PCR Rack).
- Add 50~75 µl of RNase-Free Water to the membrane center of the Filter Plate. Stand for 3 mins.
  -- Important Step! For effective elution, make sure that RNase-Free Water is dispensed on the membrane center and is absorbed completely.
  -- Important : Do not elute the DNA/RNA using RNase-Free Water less than suggested volume (<50 µl). It will lower the DNA/RNA yield.
- Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- Open the manifold valve to apply vacuum to elute DNA/RNA.
- Seal the Adhesive Film and store the DNA/RNA at -70°C.

Protocol: Centrifuge processing

Please read Important Notes and Additional Materials Required before starting the following steps.

Required hardware
Centrifuge equipment capable of 5,600~6,000 xg with a swing-bucket rotor and the adaptor for 96-well plate

STEP 1. Sample preparation and lysis
- Transfer 200 µl of sample to each well of a Collection Plate (provided, 96-well 2 ml plate; first collection plate).
  If prepared samples are less than 200 µl, adjust sample volume to 200 µl with PBS (not provided).
- Add 400 µl of VNE Buffer to each well and mix completely by pipetting.
- Incubate at room temperature for 10 minx.

STEP 2. Adjust binding condition
- Add 300 µl of AD Buffer (ethanol added) to each well and mix completely by pipetting.

STEP 3. DNA/RNA Binding
- Place a Filter Plate (provided, 96-Well nucleic acid binding plate) on a clean Collection Plate (provided, second collection plate).
- Transfer the sample mixture to each well of the Filter Plate and discard the Collection Plate (first collection plate).
- Place the plates in a rotor bucket and centrifuge at 5,600~6,000 xg for 2 mins.
- Discard the Collection Plate (second).
- Place the Filter Plate on a clean Collection Plate (provided, third Collection Plate).

STEP 4. Wash the Filter Plate with Wash Buffer 1
- Add 500 µl of Wash Buffer 1 (ethanol added) to each well of the Filter Plate.
- Place the combined plate in a rotor bucket and centrifuge at 5,600~6,000 xg for 2 mins.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

STEP 5. Wash the Filter Plate with Wash Buffer 2
- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Place the combined plate in a rotor bucket and centrifuge at 5,600~6,000 xg for 2 mins.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

STEP 6. Wash the Filter Plate again with Wash Buffer 2
- Add 500 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Centrifuge at 5,600~6,000 xg for 15 mins.
- Discard the Collection Plate (third).

STEP 7. Dry the membranes of Filter Plate
- Place the Filter Plate on top of a clean paper towel (not provided) and stand at room temperature for 10 mins.

STEP 8. DNA/RNA Elution
- Place the combined Filter Plate and Elution Plate (provided, 96-Well PCR Plate) onto the 96-Well PCR Rack (not provided), forming a three-plate assembly in the following order: top – DNA Binding Plate; middle – Elution Plate; bottom – 96-Well PCR Rack.
- Add 50~75 µl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 mins.
  -- Important Step! For effective elution, make sure that RNase-Free Water is dispensed on the membrane center and is absorbed completely.
  -- Important : Do not elute the DNA/RNA using RNase-Free Water less than suggested volume (<50 µl). It will lower the DNA/RNA yield.
- Place the combined plates in a rotor bucket and centrifuge at 5,600~6,000 xg for 5 mins to elute DNA/RNA.
- Seal the Adhesive Film and store the DNA/RNA at -70°C.