User Manual

FAVORGEN BIOTECH CORP.

FavorPrep™ 96-well Viral DNA/ RNA Extraction Kit

(For Research Use Only)

- 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)	FAVRE 96001 (1 plate)	FAVRE 96002 (2 plates)	FAVRE 96004 (4 plates)
VNE Buffer	60 ml	120 ml	120 ml x 2
AD Buffer [a] (concentrate)	5 ml	10 ml	10 ml x 2
Wash Buffer 1 * (concentrate)	55 ml	110 ml	110 ml x 2
Wash Buffer 2 ■ (concentrate)	25 ml	50 ml	50 ml x 2
RNase-Free Water	15 ml	30 ml	30 ml x 2
Filter Plate (96-Well DNA/ RNA binding plate)	1 plate	2 plates	4 plates
Collection Plate (96-Well 2 ml Plate)	3 plates	6 plates	12 plates
Elution Plate (96-Well PCR plate)	1 plate	2 plates	4 plates
Adhesive Film	2 pcs	4 pcs	8 plates

Preparation of working buffers

Add RNase-free ethanol (96~100%) to AD Buffer, Wash Buffer 1 and Wash Buffer 2 when first use.

Cat. No.	FAVRE 96001	FAVRE 96002 FAVRE 96004
[a] Ethanol for AD Buffer	40 ml	80 ml
* Ethanol for Wash Buffer 1	10 ml	20 ml
■ Ethanol for Wash Buffer 2	100 ml	200 ml

Quality control

The quality of 96-Well Viral DNA/ RNA Extraction Kit is tested on a lot-to-lot basis. The purified nucleic acid is checked by real-time PCR and capillary electrophoresis,

Specification:

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

Reagent and equipments to be provided by user

- 96 ~100 % RNase - free ethanol (for preparation of working solution)
- For centrifugation protocol: A centrifuge is required, capable of 5,600 ~ 6,000 X g, with a swing -bucket rotor and the adaptor for 96-well plates.
- For vacuum protocol: A vacuun manifold for 96-well plate and a vaccum source reached to 15 inches Hg are required. ***(Alternative)***: If using centrifugation for Elution Step (STEP 6), a centrifuge equiment is required, capable of 5,600 ~ 6,000 X g, with swing -bucket rotor and the adaptor for 96-well plate.

Related products can be ordered from Favorgen

	Description:
Vacuum manifold *(Cat. No: Wel-Vac 200)*	Size: 23.2x12.4x10.2 cm; material: anodized aluminum
Oil -less vacuum pump *(Cat. No: FAPMP 110/220)*	FAPMP 110: 110V, 60Hz, FAPMP 220: 220V, 50Hz, Max. vacuum: -26.8 inches Hg (-680 mm Hg)

Important Notes:

1. Make sure the environment is RNase-free when handling RNA extraction.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Add RNase-free ethanol (96~100%) to AD Buffer, Wash Buffer 1 and Wash Buffer 2 when first use.
4. The eluted RNA should immediately be kept on ice. For long term storage freeze at -70 °C.

Safety Information:

1. VNE Buffers and Wash Buffer1 provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. ***CAUTION:*** VNE Buffers and Wash Buffer1 contain guani dinium 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

Hazard statement(s)

H302 + H312 + H332	Harmful if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye damage.
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
CAS-No. 50-01-1
EC-No. 200-002-3

Hazard statement(s)

H302 + H332	Harmful if swallowed or if inhaled.
H315	Causes skin irritation.
H319	Causes serious eye irritation.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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 5 min

● STEP 2. Adjust binding condition:

Add AD Buffer → Mix by pipetting

Centrifuge protocol or Vacuum protocol

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

centrifuge protocol

- Combind the plates.
- Transfer the sample mixture to Filter plate.
- Centrifuge at 4,500 – 6,000 x g for 2 min.

Filter Plate
Collection Plate (second collection plate)

vacuum protocol

- Fix Plates to manifold.
- Transfer the sample mixture to Filter plate.
- Apply 10 inches Hg vacuum until the wells have emptied.

Manifold lid
Filter Plate
Collection Plate (second collection plate)
vacuum manifold

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

- Add Wash Buffer 1. Centrifuge at 4,500 – 6,000 x g for 2 min.

- Add Wash Buffer 1. Apply vacuum at 10 inches Hg.

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

- STEP 5: Add Wash Buffer 2. Centrifuge at 5,600 - 6,000 x g for 2 min
- STEP 6: Add Wash Buffer 2. Centrifuge at 5,600 - 6,000 x g for **15 min**

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

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

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

- 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 min.

● STEP 8. RNA Elution:

- Add RNase-free Water to the Filter Plate. Stand for 3 min.
- Centrifuge to elute RNA.

Filter Plate
PCR Plate
Collection Plate (third collection plate)

- Add RNase-free Water to the Filter Plate. Stand for 3 min.
- Close the manifold volve. Turn on the vacuum source to build up a vacuum to 15 inches Hg.
- Open the manifold volve to apply vacuum to elute DNA/ RNA.

Alternative : If the consistent volume of elutes are recommend use centrifuge protocol to proceed this elution step. **(Page 3, STEP 8)**

Filter Plate
Manifold lid
PCR Plate
Collection Plate (third collection plate)

Protocol: (centrifugation processing)

Please Read Important Notes Before Starting The Following Steps.

Required hardware

Centrifuge equiment capable of 5,600 ~ 6,000 X g 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 min.

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 x g for 2 min.
- Discard the flow-through and return the Filter Plate to the 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 x g for 2 min.
- 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 x g for 2 min.
- 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 x g for **15 min**.
- Discard the flow-through and the Collection Plate (second plate).

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 min.

STEP 8. DNA/ RNA Elution

- Place a Elution Plate (provided, 96-Well PCR plate) on top of a clean Collection Plate (provided, third collection plate) then place the Filter Plate on the Elution plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: Collection Plate)
- Add 50 ~ 75 µl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.

-- 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 plates in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 5 min to elute DNA/ RNA.
- Seal the Adhesive Film and store the RNA at -70 °C.

Protocol: (vacuum processing)

Please Read Important Notes Before Starting The Following Steps.

Required hardware

Vacuun manifold for 96-well plate and vaccum source reached to -15 inches Hg

Alternative: If using centrifugation for Elution Step (STEP 6), a centrifuge equiment is required, capable of 5,600 ~ 6,000 X g, 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 min.

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 10 inches Hg until the wells have emptied.
- Discard the flow-through and return the Filter Plate and the 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 10 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 10 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 10 inches Hg for **10 min**.
- 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 min.
- Discard the flow-through and the Collection Plate (second plate).

STEP 8. DNA/ RNA Elution

- Place a Elution Plate (provided, 96-Well PCR plate) on top of a clean Collection Plate (provided, third collection plate) and fix plates on the rack of manifold. Cover the manifold lid and place the Filter Plate on the Elution Plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: Collection Plate)
- Add 50 ~ 75 µl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.

-- 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 volve. Turn on the vacuum source to build up a vacuum to 15 inches Hg.
- Open the manifold volve to apply vacuum to elute DNA/ RNA.
- Seal the Adhesive Film and store the RNA at -70 °C.

Alternative : If the consistent volume of elutes are recommend use centrifuge protocol to proceed this elution step.