User Manual

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 nucleic acid 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.

	FAVRE 96001	FAVRE 96002	FAVRK 96004
[a] Ethanol volume for AD Buffer	40 ml	80 ml	
* Ethanol volume for Wash Buffer 1	10 ml	20 ml	
■ Ethanol volume for Wash Buffer 2	100 ml	200 ml	

Related products can be ordered from Favorgen

	Cat. No:	Description:
Vacuum manifold	Wel-Vac 200	Size: 23.2x12.4x10.2 cm; material: anodized aluminum Unique designed column adaptor board and luer connector make Wel-Vac 200 highly compatible with various kinds of centrifuge tube and 96-well plate.
Oil-less vacuum pump	FAPMP 110/220	FAPMP 110: 110V, 60Hz, Max. vacuum -26.8 inches Hg (-680 mm Hg) FAPMP 220: 220V, 50Hz, Max. vacuum -26.8 inches Hg (-680 mm Hg)

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 to be provided by user

96 ~100 % RNase - free ethanol

Important notes:

1. Make sure everything is RNase-free when handling this kit.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when andling these buffers.
3. Add RNase-free ethanol (96~100%) to AD Buffer, Wash Buffer 1 and Wash Buffer 2 when first use.
4. Equipments required:
   - 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 8), 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.

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:

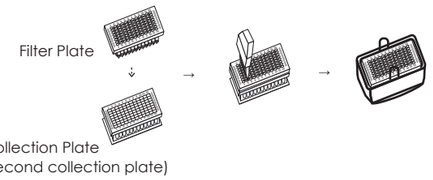
 Add AD Buffer →  Mix by pipetting

Centrifuge protocol ↙ or ↘ Vacuum protocol

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

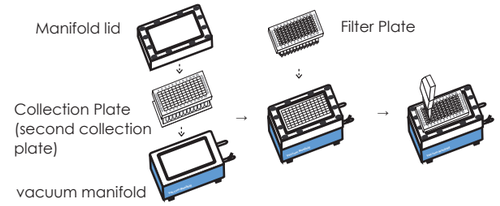
centrifuge protocol

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



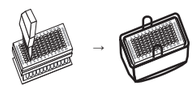
vacuum protocol

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



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

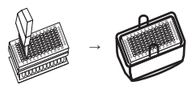
- Add Wash Buffer 1. Centrifuge at 4,500 – 6,000 x g for 2 min.



- 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**.

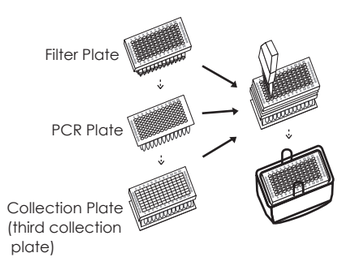
• STEP 7. Dry the membranes of the Filter Plate:

- 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.

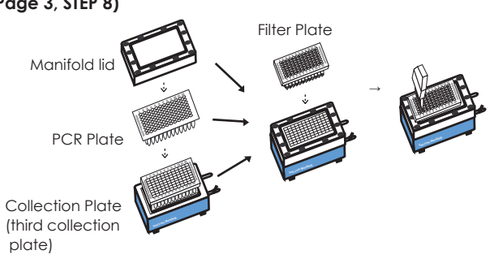
• STEP 8. RNA Elution:

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



- 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)



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 8), 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. (Page 3, STEP 8)