User Manual

FavorPrep™ 96-well PCR Clean-Up Kit

- For 96-well high-throughput purification of DNA from PCR mixture or other enzymatic reaction mixture

(For Research Use Only)

Kit contents:

Cat. No.: (Q'ty)	FACKE 96001 (1 plate)	FACKE 96002 (2 plates)	FACKE 96004 (4 plates)
FAPC Buffer	60 ml	120 ml	120 ml x 2
Wash Buffer ■ (concentrate)	15 ml	30 ml	30 ml x 2
Elution Buffer	15 ml	30 ml	30 ml x 2
Filter Plate (96-Well DNA 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 ethanol (96~100%) to Wash Buffer when first use.

Cat. No.	FACKE 96001	FACKE 96002 FACKE 96004
■ Ethanol for Wash Buffer 2	60 ml	120 ml

Quality control

The quality of 96-Well PCR Clean-Up Kit is tested on a lot-to-lot basis. The purified DNA is checked by real-time PCR and capillary electrophoresis.

Specification:

Principle: Filter Plate (silica membrane)
Sample size: 10 ~ 100 µl of PCR mixture or other enzymatic reaction mixture.
DNA size: 65 bp ~ 10 kbp
Processing: centrifugation protocol or vacuum & centrifugation protocol
Operation time: < within 45 min/ 96 preparations
Recovery: 85% ~ 95% for PCR clean-up
DNA Binding capacity: up to 15 µg/ well
Elution volume: 50 ~ 75 µl
Downstream application: Fluorescent or radioactive sequencing, Restriction digestion, Library screening, Ligation, Labeling, Transformation.

Reagent and equipments to be provided by user

- 96 ~100 % ethanol (for preparation of Wash Buffer).
- 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 5), 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. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add ethanol (96~100%) Wash Buffer when first use.
3. Check FAPC Buffer before use, Warm Lysis Buffer at 60 °C for 5 minutes if any precipitate formd.
4. Components of this kit should be stored at 15 ~ 25 °C.

Safety Information:

1. FAPC Buffers and Wash Buffer1 provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

Kit Component: FAPC, **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

Transfer samples in a Collection Plate (firet collection plate) → Add FAPC Buffer → Mix by pipetting

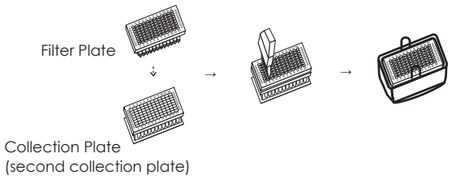
↓

Centrifuge protocol ↙ or ↘ Vacuum protocol

• STEP 2. Bind DNA to Filter Plate:

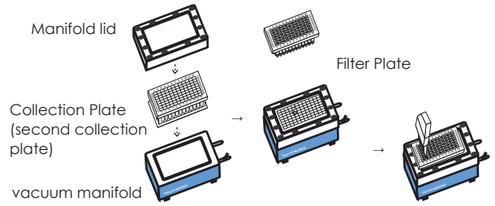
centrifuge protocol

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



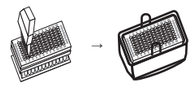
vacuum protocol

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



• STEP 3. Wash the Filter Plate with Wash Buffer

- STEP 3:
  Add Wash Buffer. Centrifuge at 5,600 - 6,000 x g for **15 min**



- STEP 3:
  Add Wash Buffer. Apply vacuumat at 10 inches Hg funtil the wells have emptied.

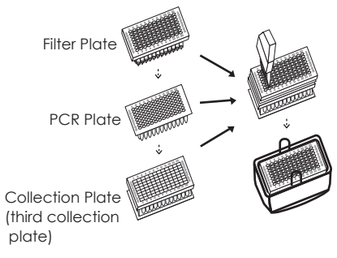
• STEP 4. Dry the membranes of the Filter Plate:

- 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.

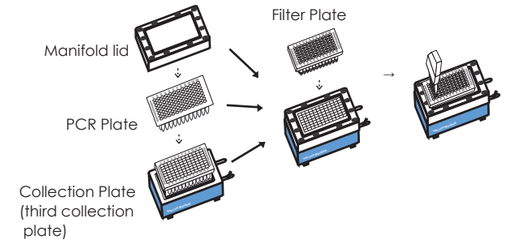
• STEP 5. RNA Elution:

- Add Elution Buffer to the Filter Plate. Stand for 3 min.
- Centrifuge to elute DNA.



- Add Elution Buffer 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.

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 10~100 µl of sample to each well of a Collection Plate (provided, 96-well 2 ml plate; first collection plate).
- Add 5 volumes of FAPC Buffer to each well and mix completely by pipetting.
  -- For example, add 500 µl of FAPC Buffer to 100 µl of sample.

STEP 2. DNA Binding

- Place a Filter Plate (provided, 96-Well DNA 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 3. Wash the Filter Plate with Wash Buffer

- Add 500 µl of Wash Buffer (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 15 min.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

STEP 4. 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 5. 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 using Elution Buffer water less than suggested volume (< 50 µl). It will lower the DNA yield.

- Place the plates in a rotor bucket and centrifuge at 5,600 – 6,000 x g for 5 min to elute DNA.
- Seal the Adhesive Film and store the DNA at -20 °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 5), 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 10~100 µl of sample to each well of a Collection Plate (provided, 96-well 2 ml plate; first collection plate).
- Add 5 volumes of FAPC Buffer to each well and mix completely by pipetting.
  -- For example, add 500 µl of FAPC Buffer to 100 µl of sample.

STEP 2. DNA 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 DNA 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 3. Wash the Filter Plate with Wash Buffer

- Add 500 µl of Wash Buffer (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 4. 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 Elutopn Buffer to the membrane center of the Filter Plate. Stand for 3 min.

-- Important Step! For effective elution, make sure that Elution Buffer is dispensed on the membrane center and is absorbed completely.

-- Important : Do not elute the DNA Elution Buffer less than suggested volume (< 50 µl). It will lower the DNA 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.
- Seal the Adhesive Film and store the DNA at -20 °C.

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