FavorPrep™ 96-Well Plasmid Kit

(For Research Use Only)

Cat.No. : FAPWE 96001, 1 Plate
FAPWE 96002, 2 Plates
FAPWE 96004, 4 plates

Kit Contents:

	FAPWE 96001 (1 plate)	FAPWE 96002 (2 plates)	FAPWE 96004 (4 plates)
FAPD1 Buffer	30 ml	65 ml	130 ml
FAPD2 Buffer	30 ml	65 ml	130 ml
FAPD3 Buffer	40 ml	75 ml	175 ml
Wash Buffer (concentrate)	15 ml †	35 ml ††	15 ml † + 55 ml †††
Elution Buffer	15 ml	30 ml	65 ml
RNase A	3 mg	6.5 mg	13 mg
Filter Plate (96-Well Plasmid plate)	1 plate	2 plates	4 plates
96-Well 2 ml Plate	3 plates	6 plates	12 plates
96-Well PCR plate	1 plate	2 plates	4 plates
Adhesive Film	2 pcs	3 pcs	6 plates

† Add 60 ml of ethanol (96-100%) to each Wash Buffer when first use.
†† Add 140 ml of ethanol (96-100%) to each Wash Buffer when first use.
††† Add 220 ml of ethanol (96-100%) to each Wash Buffer when first use.

Quality Control

The quality of 96-Well Plasmid Kit is tested on a lot-to-lot basis. The purified Plasmid is checked byagarose gel analysis and quantified with spectrophotometer.

Specification:

Principle: 96- well DNA Binding Plate (silica membrane)
Sample size: 1~ 5 ml *E coli* / preparation
Processing: centrifugation protocol or vacuum & centrifugattion protocol
Operation time: < within 1 hour/ 96 preparation
DNA Binding capacity: up to 25 µg/ well
Elution volume: 100 ~200 µl
DNA recovery rate: 80 ~ 90 %

Important Notes:

1. Buffers provided in this kit contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check FAPD2 Buffer before use, Warm the FAPD2 Buffer at 60 °C for 5 minutes if any precipitate formd.
3. Store RNase A at -20 °C upon recipit of kit.
4. Add 0.5 ml of FAPD1 Buffer to a RNase A tube and vortex the tube to mix well. Transfer the total RNase A mixture back to the FAPD1 bottle and mix well by vortexing. Store the FAPD1 buffer at 4 °C.
5. Add required ethanol (96-100%) to Wash Buffer before use.

Brief procedure:

Material to be provided by user for a 96-well preparation

1. Centrifuge equiment with a swing -bucket rotor and adaptor for 96-well plate, capable of at least 5,600 ~ 6,000 X g.
2. Vacuun manifold for 96-well plate and a vaccum source
3. 96 ~100 % ethanol

- **Resuspension** — Add 250 µl of FAPD1 Buffer to cell pellet → Resuspend the cell by pipetting
- **Lysis** — Add 250 µl of FAPD2 Buffer → Mix well by pipetting → Stand for 2~5 min

- **Neutralization** — Add 350 µl of FAPD3 Buffer → Mix well by pipetting → Seal with Adhesive Film. Place in a rotor bucket and centrifuge at 4,500 – 6,000 x g, 10 minutes. or

- **Bind plasmid to Filter Plate:**

centrifuge protocol

· Assembly plates
· Transfer the clear lysates to the Filter Plate.
· Place the combined plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.

Filter Plate → transfer the lysates to the Filter Plate → centrifuge
second 96-well 2 ml plate

vacuum protocol

· Place the plates on the rack of vacuum manifold.
· Transfer the clear lysates from the first 96-well 2 ml plate to the Filter Plate
· Apply vacuum until the wells have emptied.

Manifold lid, Filter Plate, second 96-well 2 ml plate, vacuum manifold → transfer the lysates to the Filter Plate and apply vacuum

- **Wash Filter Plate:**

· Add Wash Buffer (ethanol added) to each well of the Filter Plate.
· Place the Filter plate combined with the second 96-well plate in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 15 min.

add Wash Buffer → centrifuge

· Add Wash Buffer (ethanol added) to each well of the Filter Plate.
· Apply vacuum until the wells have emptied.

add Wash Buffer and apply maximum vacuum

- **Dry the membranes of Filter Plate:**

· Place the combined plate at 70 °C for 10 min.

· Apply maximum vacuum for an addition 10 minutes.

- **Plasmid Elution:**

· Assembly plates.
· Add Elution Buffer or ddH2O to the Filter Plate. Stand for 3 minutes.
· Centrifuge at 4,500 – 6,000 x g for 5 minutes to elute plasmid.

Filter Plate, PCR Plate, third 96-well 2 ml plate → Add Elution Buffer to the Filter Plate → centrifuge

· Gently tap the tips of the Filter Plate on paper towel to remove residual Wash Buffer.
· Assembly and place the plates on the rack of vacuum manifold .
· Add Elution Buffer or ddH2O to the Filter Plate. Stand for 3 minutes.
· Apply maximum vacuum for 5 minutes to elute plasmid

Manifold lid, Filter Plate, PCR Plate, third 96-well 2 ml plate → Add Elution Buffer to the Filter Plate. Apply vacuum.

Protocol: (centrifugation processing)
- using centrifuging force to handle DNA binding step and washing steps.

Please Read Important Notes Before Starting The Following Steps.

Material to be provided by user for a 96-well preparation

1. Centrifuge equiment with a swing -bucket rotor and adaptor for 96-well plate, capable of at least 5,600 ~ 6,000 X g.
2. 96 ~100 % ethanol

STEP 1
Resuspend the cells
- Resuspend bacterial cultures in 250 µl of FAPD1 Buffer (RNase A added) and transfer to the first 96-well 2 ml plate (provided).

  Note: Complete cells resuspension is important for the lysis step.

STEP 2
Lysis
- Add 250 µl of FAPD2 Buffer. Mix immediately by pipetting.
- Stand for 2~5 minutes at room temperature until lysate clear.

STEP 3
Neutralization
- Add 350 µl of FAPD3 Buffer. Mix immediately by pipetting. Seal with Adhesive Film.
- Place te plate in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 10 minutes.

  Important Note: make sure that buffers have been mixed completely.

STEP 4
DNA Binding
- Place a Filter Plate (96-Well Plasmid Plate) on top of the second 96-Well 2 ml Plate (provided).
- Remove the Adhesive Film from the first 96-well 2 ml plate. Transfer the clear lysate (supernatant) to the Filter Plate.
- Place the combined plate (Filter Plate combined with the second 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.
- Discard the flow-through and return the Filter Plate to the second 96-Well 2 ml Plate.

STEP 5
Wash
- Add 650 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- Place the combined plate (Filter Plate combined with the second 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 15 min.
- Discard the second 96-Well 2 ml plate and the flow-through.

STEP 6
Dry the membranes of Filter Plate
- Place the Filter Plate on top of the third 96-Well 2 ml plate (provided) and incubate at 65 °C for 10 min

STEP 7
Plasmid Elution
- Apart the combined plate of step 6.
- Place a 96-well PCR Plate (provided) on top of the third 96-Well 2 ml Plate and place the Filter Plate on the 96-Well PCR plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- Add 100 ~ 200 µl of Elution Buffer or ddH2O (pH8.0-8.5) to the membrane center of the Filter Plate. Stand for 3 minutes until Elution Buffer or ddH2O has been absorbed by the membrane completely.
- Place the combined plate in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g for 5 min to elute plasmid to the 96-well PCR plate.

Protocol: (Vacuum processing)
- using vacuum force to handle DNA binding step and washing steps.

Please Read Important Notes Before Starting The Following Steps.

Material to be provided by user for a 96-well preparation

1. Centrifuge equiment with a swing -bucket rotor and adaptor for 96-well plate, capable of at least 5,600 ~ 6,000 X g.
2. Vacuun manifold for 96-well plate and a vaccum source.
3. 96 ~100 % ethanol

STEP 1
Resuspend the cells
- Resuspend bacterial cultures in 250 µl of FAPD1 Buffer (RNase A added) and transfer to the first 96-well 2 ml plate (provided).

  Note: Complete cells resuspension is important for the lysis step.

STEP 2
Lysis
- Add 250 µl of FAPD2 Buffer. Mix immediately by pipetting.
- Stand for 2~5 minutes at room temperature until lysate clear.

STEP 3
Neutralization
- Add 350 µl of FAPD3 Buffer. Mix immediately by pipetting. Seal with Adhesive Film.
- Place te plate in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 10 minutes.

  Important Note: make sure that buffers have been mixed completely.

STEP 4
DNA Binding
- Place the scond 96-Well 2 ml plate (provided) on the rack of vacuum manifold and cover the manifold lid. And place a Filter Plate (96-well plasmid Plate, provided) on top of the second 96-Well 2 ml plate.
- Remove the Adhesive Film from the first 96-well 2 ml plate. Transfer the clear lysate (supernatant) to the Filter Plate.
- Apply vacuum until the wells have emptied.

STEP 5
Wash
- Add 650 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- Apply vacuum until the wells have emptied.

STEP 6
Dry the membranes of Filter Plate
- Apply maximum vacuum for an addition 10 minutes to dry the membranes of Filter Plate.
- Discard the second 96-Well 2 ml plate and the flow-through.

STEP 7
Plasmid Elution
- Gently tap the tips of the Filter Plate on paper towel to remove residual Wash Buffer.
- place a 96-well PCR Plate (provided) on top of the third 96-Well 2 ml Plate (provided). Place the combined plate on the rack of vacuum manifold and cover the manifold lid.
- place the Filter Plate on top of the 96-well PCR Plate.
- Add 100 ~ 200 µl of Elution Buffer or ddH2O on the membrane center of the Filter Plate. Stand for 3 minutes.
- Apply maximum vacuum for 5 minutes to elute plasmid to the 96-well PCR plate.