User Manual

FavorPrep™ 96-Well Plasmid Kit

(For Research Use Only)

- For 96-well high-throughput extraction of plasmid from 1 ~ 5 ml overnight culture

Kit contents:

Cat. No.: (Q'ty)	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	85 ml	175 ml
Wash Buffer (concentrate)	15 ml■	35 ml▲	35 ml▲x 2
Elution Buffer	15 ml	30 ml	65 ml
RNase A (lyophilized)	3 mg	6.5 mg	13 mg
Filter Plate (96-Well Plasmid 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	4 pcs	8 pcs	16 pcs

■,▲: Add ethanol (96 ~ 100%) to Wash Buffer when first use.

Storage:

1. Kit components except RNase A and FAPD1 Buffer should be stored at room temperature (15 ~ 25°C) .
2. RNase A should be stored at -20°C upon receipt.
3. After adding RNase A, FAPD1 Buffer should be stored at 4 ~ 8°C.

Quality control

The quality of FavorPrep™ 96-Well Plasmid Kit is tested on a lot-to-lot basis. The plasmid is checked by restriction enzyme digestion and optical density ratio 260/280.

Specification:

Principle: Filter Plate (96-well plate, glass fiber membrane)
Sample size: 1~ 5 ml culture / preparation
Processing: vacuum or centrifugation
Operation time: < within 60 min/ 96 preparations
Plasmid Binding capacity: up to 60 µg/ well
Elution volume: 50 ~ 75 µl

Product description:

FavorPrep™ 96-well Plasmid Kit is designed for 96 wells high-throughput isolation of plasmid. The technology is based on alkaline lysis followed by adsorption of DNA onto silica membrane in the presence of high salt. Plasmid DNA purified with this product is immediately ready for use. High-quality plasmid DNA is eluted in a small volume of Elution Buffer or deionized water. Plasmid prepared by FavorPrep 96-well Plasmid Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro translation, and transfection of robust cells.

Important note:

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. Add RNase A to FAPD1 Buffer when first use. (see Preparation of working buffers)
4. Add ethanol (96 ~ 100%) to Wash Buffer when first use. (see Preparation of working buffers)

Additional materials required

For All Protocol:

- Pipets and pipet tips, sterile
- 96 ~100 % ethanol (for preparation of Wash Buffer).

For vacuum processing:

- A centrifugator is required for the clarification of lysate and for the alternative of elution step, capable of 5,600 ~ 6,000 X g, with a swing -bucket rotor and the adaptor for 96-well plates.
- A vacuum manifold for 96-well plate and a vacuum source reached to -12 inches Hg are required.

For centrifuge processing:

- A centrifugator is required, capable of 5,600 ~ 6,000 X g, with a swing-bucket rotor and the adaptor for 96-well plates.

Preparation of working buffers:

1. **Working PDE1 Buffer**
   Add 0.5 ml of FAPD1 Buffer to lyophilized RNase A and vortex the tube to dissolve the RNase A well. Transfer the mixture back to the FAPD1 bottle and store at 4°C.
2. **Working Wash Buffer**
   Add 96~100 % ethanol to Wash Buffer when first use. Store the buffers at room temperature (15 ~ 25 °C).

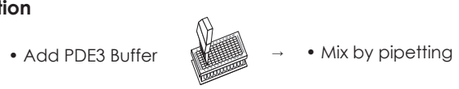
Cat. No. /	FAPWE 96001	FAPWE 96002, FAPWE 96004
Ethanol for Wash Buffer	■ 60 ml	▲140 ml

Brief procedure:

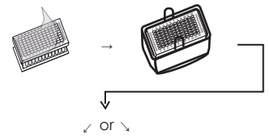
• STEP 1. Collect bacterial cells and resuspend the cells

• STEP 2. Lysis

• STEP 3. Neutralization



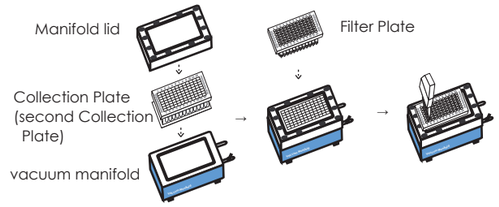
• STEP 4. Clarify lysate



• STEP 5. Bind plasmid to Filter Plate:

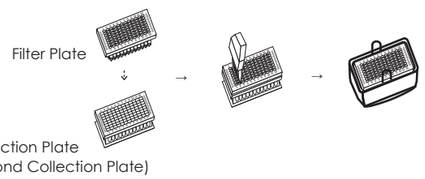
Vacuum processing

- Transfer the supernatant to Filter plate.
- Apply -12 inches Hg vacuum until the wells have emptied.



Centrifuge processing

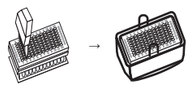
- Transfer the supernatant to Filter plate.
- Centrifuge at 4,500 ~ 6,000 x g for 2 min.



• STEP 6. Wash the Filter Plate and dry the membrane of the Filter Plate

- Add Wash Buffer.
- Apply vacuum at -12 inches Hg for 2 min.
- Tap the Filter Plate tips on paper towel
- Return the Filter Plate and the Collection Plate to the manifold.
- Apply vacuum at -12 inches Hg for an additional **10 min**.

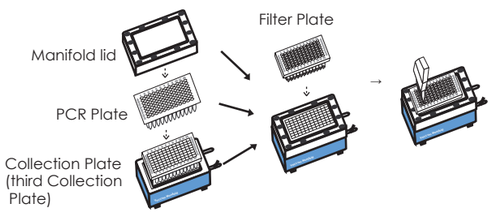
- Add Wash Buffer.
- Centrifugeat 5,600 ~ 6,000 x g for **10 min**
- Stand the Filter plate on a clean paper towel at room temperature for **5 min**.



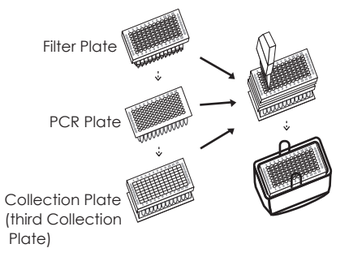
• STEP 7. Plasmid Elution:

- Add Elution Buffer or ddH2O to the Filter Plate. Stand for 3 min.
- Close the manifold volve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- Open the manifold valve to apply vacuum to elute plasmid.

Alternative : If the consistent volume of elutes are recommend, use centrifuge protocol to process this elution step. (STEP 7-A ~7-D).



- Add Elution Buffer or ddH2O to the Filter Plate. Stand for 3 min.
- Centrifuge to elute plasmid.



Safety Information:

1. PDE2 Buffer and PDE3 Buffer provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. ***CAUTION:*** PDE3 Buffers contain guanidinium 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: **FAPD2 Buffer**

Hazard contents
Sodium hydroxide
CAS-No. 1310-73-2
EC-No. 215-185-5

WARNING

Hazard statement(s)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.

Precautionary statement(s)

P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
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: **FAPD3 Buffer**

Hazard contents
Guanidine hydrochloride
CAS-No. 50-01-1
EC-No. 200-002-3

WARNING

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.

Kit Component: **RNase A**

Hazard contents
RNase A
CAS-No. 65742-22-5
EC-No. 232-646-6

DANGER

Hazard statement(s)

H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statement(s)

P261	Avoid breathing {dust/fume/gas/mist/ vapors/spray}.
P285	In case of inadequate ventilation wear respiratory protection

Response Statement(s)

P304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P342+311	If experiencing respiratory symptoms call a POISON CENTER or doctor/ physician

Protocol: Vacuum processing

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

STEP 1. Collect bacteria cells and resuspend the cells

1-1. Transfer up to 2 ml bacterial culture to each well of a Collection Plate. (provided, 96-well 2 ml plate; first Collection Plate).
1-2. Seal with a Adhesive Film on the Collection Plate. Place the plate in a rotor bucket and centrifuge at 5,600 ~ 6,000 x g for 3 min. Repeat step 1-1 & 1-2 if more than 2 ml culture should be collected.
Note: Do not exceed culture 5 ml culture.
1-3. Add 250 µl of FAPD1 Buffer (RNase A added) and resuspend the cells by pipetting.
Note: Make sure that the cells be thoroughly resuspended.

STEP 2. Lysis

2-1. Add 250 µl of FAPD2 Buffer. Mix immediately by gently pipetting the sample mixture 5 times.
2-2. Stand for 3 ~ 5 min at room temperature until lysate clear.

STEP 3. Neutralization

3-1. Add 350 µl of FAPD3 Buffer. Mix immediately by pipetting.
Note: make sure that buffers have been mixed completely.

STEP 4. Clarify lysate

4-1. Seal with a new Adhesive Film. Place the plate in a rotor bucket and centrifuge at 4,500 ~ 6,000 xg for 10 min.

STEP 5. Bind plasmid to Filter Plate

5-1. 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 Plasmid binding plate) on top of the second Collection Plate.
5-2. Transfer the sample mixture to the Filter Plate and discard the first Collection Plate.
5-3. Apply vacuum at -12 inches Hg until the wells have emptied.
5-4. Release vacuum from the manifold.
5-5. Discard the flow-through. Return the Filter Plate and the second Collection Plate back to the manifold.

STEP 6. Wash the Filter Plate and dry the membrane of the Filter Plate

6-1. Add 650 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
6-2. Apply vacuum at -12 inches Hg for 2 min.
6-3. Release vacuum from the manifold and discard the flow-through. Return the Filter Plate and the second Collection Plate back to the manifold.
6-4. Gently tap the tips of the Filter Plate on a clean paper towel to remove residual liquid. Return the Filter Plate to the second Collection Plate fixed in the manifold.
6-5. Apply vacuum at -12 inches Hg for an addition **10 min**.
6-6. Release vacuum from the manifold and discard the flow-through and the third Collection Plate.

STEP 7. Plasmid Elution

Alternative: If the consistent volume of eluates are recommended, use "centrifuge processing step 7-A ~ 7-D", to proceed this elution.

7-1. Place an 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: third Collection Plate)
7-2. Add 50 ~ 75 µl of Elution Buffer or ddH_2O to the membrane center of the Filter Plate. Stand for 3 min.
-- Note! The eluates averaged about 25 µl less than the adding volume of elution buffers. For example, adding 50 µl of Elution Buffer will recover ~25 µl of eluate.
-- Note! Do not use Elution Buffer or ddH_2O less than the suggested volume (< 50 µl). It will lower the plasmid yield.
-- Note! For effective elution, make sure that Elution Buffer or ddH_2O is dispensed on the membrane center and is absorbed completely.
7-3. Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
7-4. Open the manifold valve to apply vacuum to elute RNA.
7-5. Release vacuum from the manifold.
7-6. Take out the Elution Plate (96-well PCR plate) and seal with a Adhesive Film (provided). Store the plasmid at -20 °C before use.

Protocol: Centrifuge processing

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

STEP 1. Collect bacteria cells and resuspend the cells

1-1. Transfer up to 2 ml bacterial culture to each well of a Collection Plate. (provided, 96-well 2 ml plate; first Collection Plate).
1-2. Seal with a Adhesive Film on the Collection Plate. Place the plate in a rotor bucket and centrifuge at 5,600 ~ 6,000 x g for 3 min. Repeat step 1-1 & 1-2 if more than 2 ml culture should be collected.
Note: Do not exceed culture 5 ml culture.
1-3. Add 250 µl of FAPD1 Buffer (RNase A added) and resuspend the cells by pipetting.
Note: Make sure that the cells be thoroughly resuspended.

STEP 2. Lysis

2-1. Add 250 µl of FAPD2 Buffer. Mix immediately by gently pipetting the sample mixture 5 times.
2-2. Stand for 3 ~ 5 min at room temperature until lysate clear.

STEP 3. Neutralization

3-1. Add 350 µl of FAPD3 Buffer. Mix immediately by pipetting.
Note: make sure that buffers have been mixed completely.

STEP 4. Clarify lysate

4-1. Seal with a new Adhesive Film. Place the plate in a rotor bucket and centrifuge at 4,500 ~ 6,000 xg for 10 min.

STEP 5. Bind plasmid to Filter Plate

5-1. Place a Filter Plate (provided, 96-Well Plasmid binding plate) on a clean Collection Plate (provided, second Collection Plate).
5-2. Transfer the sample mixture to each well of the Filter Plate and discard the first Collection Plate.
5-3. Place the combined plates (Filter Plate + the second Collection Plate) in a rotor bucket and centrifuge at 5,600 ~ 6,000 x g for 2 min.
5-4. Discard the flow-through and return the Filter Plate to the second Collection Plate.

STEP 6. Wash the Filter Plate and dry the membrane of the Filter Plate

6-1. Add 650 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
6-2. Place the combined plate in a rotor bucket and centrifuge at 5,600 ~ 6,000 x g for **10 min**.
6-3. Place the Filter Plate on top of a clean paper towel (not provided) and stand at room temperature for **5 min**.

STEP 7. Plasmid Elution

7-A. 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: third Collection Plate)
7-B. Add 50 ~ 75 µl of Elution or ddH_2O to the membrane center of the Filter Plate. Stand for 3 min.
-- Note! The eluates averaged about 25 µl less than the adding volume of elution buffers. For example, adding 50 µl of Elution Buffer will recover ~25 µl of eluate.
-- Note! Do not use Elution Buffer or ddH_2O less than the suggested volume (< 50 µl). It will lower the plasmid yield.
-- Note! For effective elution, make sure that Elution Buffer or ddH_2O is dispensed on the membrane center and is absorbed completely.
7-C. Place the combined plates in a rotor bucket and centrifuge at 5,600 ~ 6,000 x g for 5 min to elute plasmid.
7-D. Take out the Elution Plate (96-well PCR plate) and seal with a Adhesive Film (provided). Store the plasmid at -20 °C before use.

Problem shooting:

Low yield

Bacterial cells were not lysed completely
- Too many bacterial cells were used.
- After FAPD3 Buffer addition, break up the precipitate by pipetting to ensure higher yield.

Overgrown of bacterial cells
- Incubation time should not longer than 16 hours.

Bacterial cells were insufficient
- Ensure that bacterial cells have grown to an expected amount (OD600 > 1) after incubation under suitable shaking modes.

Incorrect DNA elution step
- Ensure that Elution Buffer or ddH_2O was added and absorbed to the center of the FAPD Column membrane.
- If size of DNA fragments is larger than 10 kb, use preheated Elution Buffer or ddH_2O (60 ~ 70°C) on solution step to improve the elution efficiency.

Incorrect preparation of Wash Buffer
- Ensure that the correct volume of ethanol (96 ~ 100 %) was added to Wash Buffer prior to use.

Residual ethanol in membrane because insufficient drying step.
- Ensure that the step of dry the membrane of the Filter plate has been processed.

Genomic DNA Contaminates

Lysate prepared improperly
- For the lysis step, gently pipette the sample mixture up and down to mix well after adding the FAPD2 Buffer and do not incubat longer than 5 min.
- Do Not use overgrown bacterial culture.

RNA Contaminates

Insufficiency of RNase A activity in FAPD1 Buffer because of long -term storage
- Prior to using FAPD1 Buffer, ensure that RNase A was added. If RNase A added FAPD1 Buffer is out of date, add additional RNase A into FAPD1 Buffer to a concentration of 50 µg/ ml then store 4°C.
- Too many bacterial cells were used. reduce sample volume.