

FavorPrep[™] 96-Well Plasmid Kit

- 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 p	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 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:

A260/A280: 1.7~1.8

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 25 µg/ well Elution volume: 50 ~ 75 µl

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
- 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 $5,600 \sim 6,000 \times g$, with a swing -bucket rotor and the adaptor for 96-well plates.
- A vacuun manifold for 96-well plate and a vaccum source reached to -12 inches Hg are required. (see "Ordering information for related products")

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.

Ordering information for related products:

	Description:
Vacuum manifold (Caf. No: Wel-Vac 200)	Size: 23.2x12.4x10.2 cm; Material: Construction: anodized aluminum Gasket: Ethylene propylene O-ring: Silicone
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)

Product description:

FavorPrepTM 96-well Plasmid Kit is designed for 96 wells highthroughput 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.

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 mixtute 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).

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Cat. No.	FAPWE 96001	FAPWE 96002, FAPWE 96004	
Ethanol for Wash Buffer	■ 20 ml	▲ 140 ml	

Brief procedure:

• STEP 1. Collect bacterial cells and resuspend the cells







 Seal with a Adhesive Film. and centrifuge at 5,600-6,000 x g for 3 min



• Add PDE1 Buffer



Resuspend by pipetting

STEP 2. Lysis

Add PDE2 Buffer



• Mix gently by pipetting 5 times

STEP 3. Neutralization

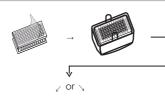
Add PDE3 Buffer



Mix by pipetting

• STEP 4. Clarify lysate

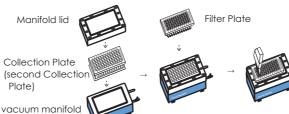
- Seal with a Adhesive Film.
 Centrifuge at 5,600 6,000 x g for 10 min



• STEP 5. Bind plasmid to Filter Plate:

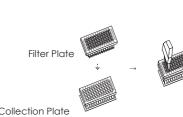
vacuum processing

 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.



Collection Plate (second Collection Plate)

• 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.



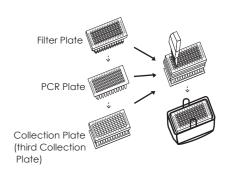


• STEP 7. Plasmid Elution:

- 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 volve 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.



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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

Hazard statement(s)

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

damage.

Precautionary statement(s)

Do not breathe dust/ fume/ gas/ mist/ P260

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.

IF INHALED: Remove person to fresh air

P304 + P340 + P310

and keep comfortable for breathing immediately call a POISON CENTER/

P305 + P351 + P338

doctor.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue

Kit Component: FAPD3 Buffer

Hazard contents

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

Hazard statement(s)

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

H319 Causes serious eye irritation.

Precautionary statement(s)

Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P261

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

Kit Component: RNase A

Hazard contents

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

Hazard statement(s) H334:

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

Precautionary statement(s)

Avoid breathing {dust/fume/gas/mist/ P261:

P285:

vapors/spray}.
In case of inadequate ventilation wear respiratory protection

Response Statement(s)

P304+340: IF INHALED: Remove victim to fresh air

ir innaleu: kemove victim to fresh air and keep at rest in a position comfortable for breathing. If experiencing respiratory symptoms call a POISON CENTER or doctor/ physician P342+311:

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 resuspensed.

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 Ha 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 ul of Wash Buffer (ethanol added) to each well of the
- 6-2. Apply vacuum at -12 inches Hg for 2 min.
- 6-3. Release vacuum from the manifold and discard the flowthrough. 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 flowthrough 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 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: third Collection Plate)
- 7-2. Add $50 \sim 75 \,\mu\text{l}$ of Elution Buffer or ddH₂O 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 ddH2O less than the suggested volume (< 50 µl). It will lower the plasmid vield.
 - -- Note! For effective elution, make sure that Elution Buffer or ddH2O is dispensed on the membrane center and is absorbed completely.

- 7-3. Close the manifold volve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- 7-4. Open the manifold volve 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
- -- Note! For effective elution, make sure that Elution Buffer or ddH2O is dispensed on the membrane center and is absorbed completely.
- 7-C. Place the combined plates in a rotor bucket and centrifuae at $5,600 - 6,000 \times g$ 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

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 a 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 resuspensed.

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 ul of Wash Buffer (ethanol added) to each well of the

- Filter Plate 6-2. Place the combined plate in a rotor bucket and centrifuae 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 $50 \sim 75 \,\mu\text{l}$ of Elution or ddH₂O 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 ddH2O less than the suggested volume (< 50 µl). It will lower the plasmid vield.

Problem shooting:

Low vield

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 arown to an expected amount (OD600 > 1) after incubation under suitable shaking modes.

Incorrect DNA elution step

- Ensure that Elution Buffer or ddH2O 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 ddH2O (60~70°C) on slution 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 pior to use.

Residual ethanol in membrane because insufficient drving 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 pipett the sample mixture up an 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.

3