User Manual

FavorPrep™ 96-Well Plant Genomic DNA Kit

(For Research Use Only)

- For 96-well high-throughput purification of pure genomic DNA from plant tissue or cultured plant cells.

Kit contents:

Cat. No.: (Q'ty)	FAPGE 96001 (1 plate)	FAPGE 96001 (2 plates)	FAPGE 96004 (4 plates)
FAPG1 Buffer	60 ml	120 ml	120 ml × 2
FAPG2 Buffer	20 ml	40 ml	80 ml
FAPG3 Buffer (concentrate)●	30 ml	60 ml	60 ml × 2
Wash Buffer (concentrate)▲	20 ml	40 ml	40 ml × 2
Elution Buffer	30 ml	60 ml	120 ml
RNase A (lyophilized)■	25 mg	50 mg	50 mg × 2
DNA Binding Plate (96-Well)	1 plate	2 plates	4 plates
Filter Plate (96-Well)	1 plate	2 plates	4 plates
Elution Plate (96-Well PCR plate)	1 plate	2 plates	4 plates
Adhesive Film	4 pcs	8 pcs	16 pcs

●, ▲, ■, ***see Working Buffer Preparation.***

Storage:

1. All kit components are shipped at room temperature, and should be stored at room temperatures between 15~25°C upon receipt, except RNase A.
2. RNase A (lyophilized) should be stored at temperatures between 2~8°C upon receipt.

Working Buffer Preparation:

1. "■" Preparation of RNase A solution (50 mg/ml)

Add required ddH_2O as the table below indicates. Vortex and make sure that RNase A has been completely dissolved. Store the RNase A stock solution at 4°C.

Cat. No.	FAPGE 96001	FAPGE 96002 FAPGE 96004
ddH_2O volume	0.5 ml	1 ml

2. "●, ▲" Preparation of FAPG3 Buffer and Wash Buffer

Add required ethanol (96~100%) as the table below indicates. Store the FAPG3 Buffer and Wash Buffer (ethanol added) at 15~25°C.

Buffer \ Cat. No.	FAPGE 96001	FAPGE 96002 FAPGE 96004
Ethanol volume for FAPG3 Buffer	60 ml	120 ml
Ethanol volume for Wash Buffer	80 ml	160 ml

Quality control

The quality of FavorPrep™ 96-Well Plant Genomic DNA Kit is tested on a lot-to-lot basis. The purified DNA is checked by agarose gel analysis and quantified with spectrophotometer.

Specification:

Sample amount:
- Up to 50 mg of fresh or frozen plant tissue
- Up to 15 mg of dry plant tissue
- Up to 5×10^6 plant cells

Operation time: About 60 minutes
Binding capacity: Up to 40 µg total DNA
Expected yield: 5~35 µg
Elution volume: 100~200 µl

Product Description:

FavorPrep™ 96-Well Plant Genomic DNA Extraction Kit is designed for high-throughput extraction of total DNA (including genomic, mitochondrial and viral DNA) from a wide variety of plant tissue and cells. The kit uses chaotropic salt to lyse cells; the DNA in chaotropic salt is bonded to glass fiber matrix of plate. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer or nuclease free water. The entire procedure can be completed in one hour without phenol/chloroform extraction and alcohol precipitation. The kits can be used for manual filtration or with robotic handing systems, the purified DNA fragment is approximately 20-30 kb which is suitable for PCR or other enzymatic reactions.

Additional materials required

For All Protocol:
1. 96-well 2.0 ml plate (2.0 ml, 96 well deep collection plate)
2. Centrifuge equipment with a swing-bucket rotor, capable of 4,500~6,000 x g
3. Water bath or incubator capable for 65°C
4. Liquid nitrogen or equipment for disrupting sample
5. Absolute ethanol (96~100%)
6. -20°C freezer

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 4,500~6,000 x g, with a swing-bucket rotor and the adaptor for 96-well plates.

Safety Information:

1. FAPG3 Buffer provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. ***CAUTION:*** FAPG3 Buffer 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: **RNase A**

Hazard contents:

Ribonuclease A (from bovine pancreas) CAS No: 9001-99-4

GHS symbol **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.
P304 + P340 + P312 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell.

Kit Component: **FAPG3 Buffer**

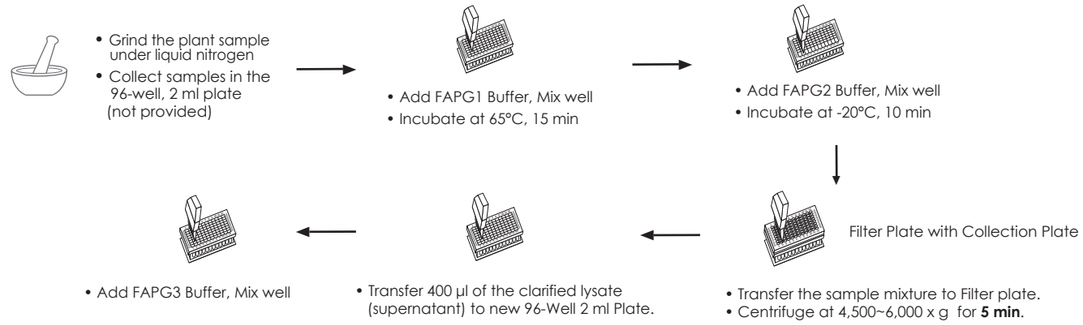
Hazardous contents
Guanidine hydrochloride, 20~50%, CAS-No. 50-01-1

GHS symbol **Warning**

Hazard statement(s)
H302 Harmful if swallowed.
H319 Causes serious eye irritation.
Precautionary statement(s)
P264 Wash thoroughly after handling.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.

Brief procedure:

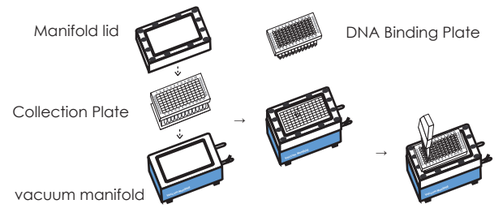
● STEP A. Sample preparation



● STEP B. Bind DNA to DNA Binding Plate:

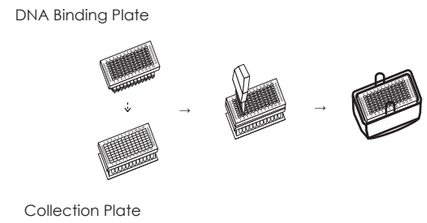
Vacuum/Centrifuge processing

- Transfer the sample mixture to DNA Binding Plate.
- Apply -12 inches Hg vacuum until the wells have emptied.



Centrifuge processing

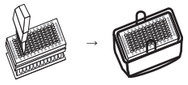
- Transfer the sample mixture to DNA Binding Plate.
- Centrifuge at 4,500~6,000 x g for **5 min**.



● STEP C. Wash the DNA Binding Plate with Wash Buffer

- Add Wash Buffer. Apply vacuumat at -12 inches for **5 min**

- Add Wash Buffer. Centrifuge at 4,500~6,000 x g for **5 min**

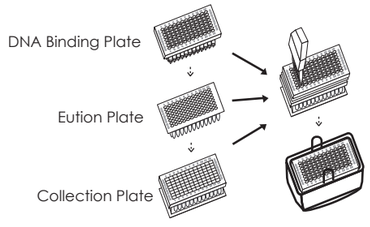


● STEP D. Dry the membranes of the DNA Binding Plate:

- Centrifuge at 4,500~6,000 x g for an additional **10 minutes**
- Combine the DNA Binding Plate with a 96-Well 2 ml Plate
- Centrifuge at 4,500~6,000 x g for **10 minutes**

● STEP E. DNA Elution:

- Add Elution Buffer to the DNA Binding Plate. **Stand for 5 min**.
- Centrifuge to elute DNA.



Important notes:

1. Buffers provided in this system contain irritants please wear gloves, lab safety goggles and laboratory coat.
2. The maximum sample size is 50 mg of tissue and 5×10^6 of cultured cells per well, do not exceed the maximum limit.
3. Prepare FAPG1/RNase A solution (400 µl FAPG1 Buffer contains 4 µl of RNase A per reaction) in quantity sufficient before the high-throughput operation.
4. For grinding the tissue sample, the homogenizer is recommended to disrupt the multiple samples for high throughput extraction.
5. Adding indicated volume of ethanol (96~100%) to FAPG3 Buffer and Wash Buffer when first open the buffer bottles.
6. Prepare a dry bath or a water baths to 65°C before experiment.

Vacuum/Centrifuge Protocol:

Please Read Important Notes and Safety Information before starting the following steps.

STEP A. Sample preparation

1. Grind the plant sample under liquid nitrogen to a fine powder, then transfer the powder to each well of a 96-Well 2 ml plate (not provided).
2. Add 400 µl of FAPG1 Buffer (RNase A added) to each well of the plate, sealing with an adhesive film and shaking the plate briefly to mix the sample.
3. Incubate the plate on 65°C for 15 min, briefly shaking the plate 3 times every 5 min during the incubation.
4. Centrifuge the plate at 4,500~6,000 x g for 1 min to remove the drops from the adhesive film, then remove and discard the adhesive film.
5. Add 130 µl of FAPG2 Buffer to each well of the plate, sealing with a new adhesive film and vortex the plate vigorously for 20 seconds to mix the sample thoroughly.
6. Place the plate to -20°C freezer for 10 min.
7. Centrifuge the plate at 4,500~6,000 x g for 1 min to remove the drops from the adhesive film, then remove and discard the adhesive film.
8. Combine a Filter Plate on another 96-Well 2 ml Plate and transfer the entire lysate from step 7 to the combined Filter Plate.
9. Place the combined plates (Filter Plate + 96-Well 2 ml Plate) in a rotor bucket and centrifuge at 4,500~6,000 x g for 5 minutes.
10. Transfer 400 µl of the clarified lysate (supernatant) from the 96-Well 2 ml Plate of Step 9 to another 96-Well 2 ml Plate.
    -- Note: Do not disrupt the pellet!
11. Add 600 µl of FAPG3 Buffer (ethanol added) to each well of the 96-Well 2 ml plate from step 10, sealing with a new adhesive film and vortex the plate vigorously for 20 seconds to mix the sample thoroughly.
12. Centrifuge the plate at 4,500~6,000 x g for 1 min to remove the drops from the adhesive film, then remove and discard the adhesive film.

STEP B. Bind DNA to DNA Binding Plate

13. Place the DNA Binding Plate on the vacuum manifold and transfer the entire lysate of each well from Step 12 to the DNA Binding Plate.
14. Apply vacuum at -12 inches Hg for 5 minutes until wells have emptied.

STEP C. Wash the DNA Binding Plate with Wash Buffer

15. Add 300 µl of Wash Buffer (ethanol added) to each well to wash the membrane of DNA Binding Plate. And apply vacuum at -12 inches Hg for 5 minutes until wells have emptied.
16. Wash again, repeat step 15.

STEP D. Dry the membranes of the Filter Plate

17. Combine the DNA Binding Plate with a 96-Well 2 ml Plate and place the combined plates (DNA Binding Plate + 96-Well 2 ml Plate) in a rotor bucket and centrifuge at 4,500~6,000 x g for an additional 10 minutes (or incubate at 65°C oven for 10 minutes) to remove residual ethanol. Discard the flow-through.

STEP E. Elution

18. Combine the DNA Binding Plate with an Elution Plate (provided). Add 100~200 µl of Elution Buffer or ddH_2O to each well of the DNA Binding Plate. Stand the combined plate (DNA Binding Plate + PCR Plate) for 5 minutes until Elution Buffer or ddH_2O has been absorbed by the membrane.
    -- 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 less than the suggested volume (< 50 µl). It will lower the DNA yield.
    -- Note! For effective elution, make sure that Elution Buffer is dispensed on the membrane center and is absorbed completely.
19. Place the combined plates (DNA Binding Plate + Eltion Plate) on a 96-Well 2 ml Plate to form a three plates complex (top: DNA Binding Plate; middle: Elution Plate; bottom: 96-Well 2 ml Plate)
20. Place the plate complex into the rotor bucket and centrifuge at 4,500~6,000 x g for 5 min to elute purified DNA into the Elution Plate.
21. Store the eluted DNA at -20°C.

Centrifuge Protocol:

Please Read Important Notes and Safety Information before starting the following steps.

STEP A. Sample preparation

1. Grind the plant sample under liquid nitrogen to a fine powder, then transfer the powder to each well of a 96-Well 2 ml plate (not provided).
2. Add 400 µl of FAPG1 Buffer (RNase A added) to each well of the plate, sealing with an adhesive film and shaking the plate briefly to mix the sample.
3. Incubate the plate on 65°C for 15 min, briefly shaking the plate 3 times every 5 min during the incubation.
4. Centrifuge the plate at 4,500~6,000 x g for 1 min to remove the drops from the caps or the adhesive film, then remove and discard the caps or the adhesive film.
5. Add 130 µl of FAPG2 Buffer to each well of the plate, sealing with a new adhesive film and vortex the plate vigorously for 20 seconds to mix the sample thoroughly.
6. Place the plate to -20°C freezer for 10 min.
7. Centrifuge the plate at 4,500~6,000 x g for 1 min to remove the drops from the adhesive film, then remove and discard the adhesive film.
8. Combine a Filter Plate on another 96-Well 2 ml Plate and transfer the entire lysate from step 7 to the combined Filter Plate.
9. Place the combined plates (Filter Plate + 96-Well 2 ml Plate) in a rotor bucket and centrifuge at 4,500~6,000 x g for 5 minutes.
10. Transfer 400 µl of the clarified lysate (supernatant) from the 96-Well 2 ml Plate of Step 9 to another 96-Well 2 ml Plate.
    -- Note: Do not disrupt the pellet!
11. Add 600 µl of FAPG3 Buffer (ethanol added) to each well of the 96-Well 2 ml plate from step 10, sealing with a new adhesive film and vortex the plate vigorously for 20 seconds to mix the sample thoroughly.
12. Centrifuge the plate at 4,500~6,000 x g for 1 min to remove the drops from the adhesive film, then remove and discard the adhesive film.

STEP B. Bind DNA to DNA Binding Plate

13. Combine a DNA Binding Plate with another 96-Well 2 ml Plate and transfer the entire lysate from step 12 to the combined DNA Binding Plate.
14. Place the combined plates in a rotor bucket and centrifuge at 4,500~6,000 x g for 5 min. Discard the flow-through and return the DNA Binding Plate to the 96-Well 2 ml Plate.

STEP C. Wash the DNA Binding Plate with Wash Buffer

15. Add 300 µl of Wash Buffer (ethanol added) to each well to wash the membrane of the DNA Binding Plate. Place the combined plates in a rotor bucket and centrifuge at 4,500 ~ 6,000 x g for 5 min. Discard the flow-through and return the DNA Binding Plate to the 96-Well 2 ml Plate.
16. Wash again, repeat step 15.

STEP D Dry the membranes of the DNA Binding Plate

17. Combine the DNA Binding Plate with a 96-Well 2 ml Plate and place the combined plates (DNA Binding Plate + 96-Well 2 ml Plate) in a rotor bucket and centrifuge at 4,500~6,000 x g for an additional 10 minutes (or incubate at 65°C oven for 10 minutes) to remove residual ethanol. Discard the flow-through.

STEP E. Elution

18. Combine the DNA Binding Plate with an Elution Plate (provided). Add 100~200 µl of Elution Buffer or ddH_2O to each well of the DNA Binding Plate. Stand the combined plate (DNA Binding Plate + Elution Plate) for 5 minutes until Elution Buffer or ddH_2O has been absorbed by the membrane.
    -- 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.
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19. Place the combined plates (DNA Binding Plate + Elution Plate) on a 96-Well 2 ml Plate to form a three plates complex (top: DNA Binding Plate; middle: Elution Plate; bottom: 96-Well 2 ml Plate)
20. Place the plate complex into the rotor bucket and centrifuge at 4,500~6,000 x g for 5 min to elute purified DNA into the Elution Plate.
21. Store the eluted DNA at -20°C.