FavorPrep™ 96-Well Plant Genomic DNA Extraction Kit

User Manual

Cat. No.: FAPGE 000 (1 Plate)
FAPGE 001 (4 Plates)
FAPGE 002 (10 Plates)

For Research Use Only

v.1001

Introduction

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 use CTAB method and chaotropic salt to lyse cells and 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 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 and purified DNA with approximately 20-30 kb is suitable for PCR or other enzymatic reactions.

Kit Contents

	FAPGE 000 (1 plate)	FAPGE 001 (4 plates)	FAPGE 002 (10 plates)
FAPG1 Buffer	45 ml	175 ml	220 ml x 2
FAPG2 Buffer	18 ml	65 ml	150 ml
FAPG3 Buffer* (concentrated)	30 ml*	60 ml* x 2	75 ml* x 2
Wash Buffer* (concentrated)	15 ml*	55 ml*	40 ml* x 4
Elution Buffer	25 ml	100 ml	120 ml x 2
RNase A (50 mg/ml)	450 µl	900 µl x 2	1100 µl x 4
DNA Binding Plate (96 well)	1 pcs	4 pcs	10 pcs
Filter Plate (96 well)	1 pcs	4 pcs	10 pcs
PCR Plate (96 well)	1 pcs	4 pcs	10 pcs
Adhesive Film	3 pcs	12 pcs	30 pcs

* Add required volume of ethanol (96~100%) to FAPG3 Buffer and Wash Buffer when first open, the required volume of ethanol (96~100%) is list as below.

	FAPGE 000	FAPGE 001	FAPGE 002
Ethanol volume for FAPG3 Buffer	60 ml	120 ml	150 ml
Ethanol volume for Wash Buffer	60 ml	220 ml	160 ml

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 throughly.

12. Centrifuge the plate at 4,500 rpm for 1 min to remove the drops from the adhesive film, then remove and discard the adhesive film.

13. Place the DNA Binding Plate on the vaccum manifold and transfer the entire lysate of each well from Step 12 to the DNA Binding Plate.

14. Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.

15. Add 300 µl of Wash Buffer (ethanol added) to each well to wash the membrane of DNA Binding Plate. And apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.

16. Wash again, add 300 µl of Wash Buffer (ethanol added) to each well to wash the membrane of the DNA Binding Plate. And apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.

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 rpm for an additional 10 minutes (or incubate at 65 °C oven for 10 minutes) to remove residual ethanol. Discard the flow-through.

18. Combine the DNA Binding Plate with a clean PCR 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.

19 Place the combined plates (DNA Binding Plate + PCR Plate) on a 96-Well 2 ml Plate to form a three plates complex (top: DNA Binding Plate, middle: PCR Plate, bottom: 96-Well 2 ml Plate)

20. Place the plate complex into the rotor bucket and centrifuge at 4,500 rpm for 5 min to elute purified DNA into the PCR Plate.

21. Store the eluted DNA at -20 °C.

Vaccum / Centrifuge Protocol:

1A. Place the plant sample to each well of a 96-Well plate which can fit the clamps of homogenizer.
2A. Add 400 µl of FAPG1 Buffer (RNase A added) and beads (not provided) to each well of the plate, sealing with a caps (not provided) and disrupt the plant sample completely by homogenizer.

1B. 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).
2B. Add 400 µl of FAPG1 Buffer (RNase A added) to each well of the plate, sealing with a adhesive film and shaking the plate briefly to mix the sample.

3. Incubate the plate on 65°C for 15 min, during the incubation briefly shaking the plate 3 times for every 5 min.

4. Centrifuge the plate at 4,500 rpm 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 throughly.

6. Place the plate to -20 °C freezer for 10 min.

7. Centrifuge the plate at 4,500 rpm 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 rpm 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 !

Quality Control:

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

Additional Requirements:

1. 96-well 2.0 ml plate (2.0 ml, 96 well deep collection plate)
2. Centrifuge equiment with a swing-bucket rotor, capable of at least 5,000 X g
3. 65 °C waterbath and 65 °C incubator
4. Liquid nitrogen or equipment for disrupting sample
5. Absolute (96~100%) ethanol
6. -20 °C freezer

Specification:

Sample Amount: up to 50 mg of fresh or forzen plant tissue
up to 15 mg of dry plant tissue
up to 5 X 10^6 plant cells
Operation time: about 60 min
Binding Capacity: up to 40 µg total DNA
Expected Yield: 5~35 µg
Elution volume: 200 µl

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. The maxium sample size is 50 mg of tissue and 1x 10^7 of cultured cells per well, do not use the sample more than this limitation.
3. Prepare a FAPG1/RNase A solution (400 µl FAPG1 Buffer : 4 µl RNase A) before the high-throughput operation.
4. For granding the tissue sample, we recommend to use the homogenizer to disrupt the mutiple sample for high throughput extraction.
5. Add required volume of ethanol (96- 100 %) to FAPG3 Buffer and Wash Buffer when first open.
6. Prepare a dry baths or a water baths to 65 °C before the operation.
7. Preheat the Elution Buffer to 65 °C for DNA elution.

Centrifuge Protocol:

1A. Place the plant sample to each well of a 96-Well plate which can fit the clamps of homogenizer.
2A. Add 400 µl of FAPG1 Buffer (RNase A added) and beads (not provided) to each well of the plate, sealing with a caps (not provided) and disrupt the plant sample completely by homogenizer.

1B. 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).
2B. Add 400 µl of FAPG1 Buffer (RNase A added) to each well of the plate, sealing with a adhesive film and shaking the plate briefly to mix the sample.

3. Incubate the plate on 65°C for 15 min, during the incubation briefly shaking the plate 3 times for every 5 min.

4. Centrifuge the plate at 4,500 rpm 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 throughly.

6. Place the plate to -20 °C freezer for 10 min.

7. Centrifuge the plate at 4,500 rpm 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 rpm 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 throughly.

12. Centrifuge the plate at 4,500 rpm for 1 min to remove the drops from the adhesive film, then remove and discard the adhesive film.

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 rpm for 5 min. Discard the flow-through and return the DNA Binding Plate to the 96-Well 2 ml Plate.

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 rpm for 5 min. Discard the flow -through and return the DNA Binding Plate to the 96-Well 2 ml Plate.

16. Wash again, 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 rpm for 5 min. Discard the flow-through and return the DNA Binding Plate to the 96-Well 2 ml Plate.

17. Place the combined plates in a rotor bucket and centrifuge at 4,500 rpm for an additional 10 minutes (or incubate at 65 °C oven for 10 minutes) to remove residual ethanol. Discard the flow-through.

18. Combine the DNA Binding Plate with a clean PCR Plate (provided). Add 100 ~ 200 µl of Elution Buffer or ddH_2O to each well of the DNA Binding Plate. Stand the combined plare (DNA Binding Plate + PCR Plate) for 5 minutes until Elution Buffer or ddH_2O has been absorbed by the membrane.

19. Place the combined plates (DNA Binding Plate + PCR Plate) on a 96-Well 2 ml Plate to form a three plates complex (top: DNA Binding Plate, middle: PCR Plate, bottom: 96-Well 2 ml Plate)

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21. Store the eluted DNA at -20 °C.