

Kit Contents:

Cat.No.	FAPGK 000 (4 preps_sample)	FAPGK 001 (50 preps)	FAPGK 001-1 (100 preps)	FAPGK 001-2 (200 preps)	FAPGK 001-3 (300 preps)
FAPG1 Buffer	2.0 ml	25 ml	55 ml	110 ml	165 ml
FAPG2 Buffer	1.0 ml	8 ml	15 ml	30 ml	45 ml
FAPG3 Buffer * (concentrate)	1.5 ml	15 ml	30 ml	60 ml	90 ml
Wash Buffer W1 * (concentrate)	0.8 ml	13 ml	26 ml	52 ml	78 ml
Wash Buffer W2 * (concentrate)	1.5 ml	15 ml	30 ml	30 ml x 2	30 ml x 3
Elution Buffer	1.5 ml	15 ml	30 ml	30 ml x 2	30 ml x 3
RNase A (lyophilized)	1.5 mg	22 mg	43 mg	43 mg x 2	43 mg x 3
Filter Column	4 pcs	50 pcs	100 pcs	200 pcs	300 pcs
FAPG Column	4 pcs	50 pcs	100 pcs	200 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	400 pcs	600 pcs
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* Preparation of FAPG1 Buffer, Wash Buffer W1 and W2 for first use:

Cat. No:	FAPGK 000 (4 preps)	FAPGK 001 (50 preps)	FAPGK 001-1 (100 preps)	FAPGK 001-2 (200 preps)	FAPGK 001-3 (300 preps)
ethanol for FAPG3 Buffer	3 ml	30 ml	60 ml	120 ml	180 ml
ethanol for Wash Buffer W1	1.0 ml	17 ml	34 ml	68 ml	102 ml
ethanol for Wash Buffer W2	6 ml	60 ml	120 ml	120 ml	120 ml

Specification:

Principle: spin column (silica membrane)

Sample: wet weight ≤ 100 mg

dry weight ≤ 20 mg

Operation time: < 60 min

DNA Yield: 5~40 µg

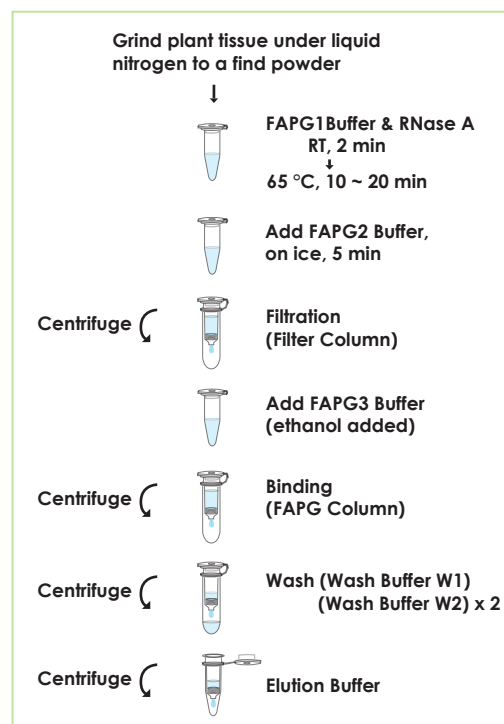
Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check FAPG1 Buffer before use, Warm FAPG1 Buffer at 60°C for 5 min if any precipitate formed.
3. Preheat dry baths or water baths to 65°C before the operation.
4. Add required ethanol (96-100%) to FAPG3 Buffer, Wash Buffer W1 and Wash Buffer W2 before use.
5. Store RNase A at -20 °C upon receipt of kit. Add sterile ddH₂O to RNase A tube to make a 50 mg/ml stock solution. Vortex and make sure that RNase A has been completely dissolved. Store the stock solution at -20 °C

General Protocol:

HINT: Prepare a 65 °C bath and a ice box for step 2 and 3.
Preheat Elution Buffer to 65 °C for step 13 (elution step).

1. Grind 50 mg of wet weight (up to 100 mg) plant tissue or 20 mg dry weight of plant tissue under liquid nitrogen to a fine powder and transfer to a new microcentrifuge tube (not provided).
• Do not allow the sample to thaw, and continue immediately to step 2.
2. Add 400 µl of FAPG1 Buffer and 8 µl of RNase A stock solution (50 mg/ml) to the tissue powder and vortex vigorously. Incubate the mixture at room temperature for 2 minutes then at 65°C for 10~20 minutes and invert 2-3 times during incubation.
3. Add 130 µl of FAPG2 Buffer to the mixture. Vortex to mix well and incubate the mixture on ice for 5 min.
4. Place a Filter Column to a Collection Tube and transfer the entire mixture from previous step to the Filter Column. Centrifuge the Filter Column at full speed (~ 18,000 x g) for 3 min.
5. Transfer the clarified lysate (supernatant) from the Collection Tube to a new microcentrifuge tube (not provided). Discard used Filter Column and Collection Tube. And adjust the volume of clarified lysate.
• Note! Do not aspirate any debris when transferring the clarified lysate.



6. Add 1.5 volume of FAPG3 Buffer (ethanol added) to the clarified lysate and mix well by pipetting.
 - Make sure that ethanol (96~100%) has been added to FAPG3 Buffer when first use.
7. Place a FAPG Column to a new Collection Tube and transfer up to 750 µl of the sample mixture from previous step carefully to the FAPG Column. Centrifuge at full speed (18,000 x g or 14,000 rpm) for 1 min. Discard the flow-through and place the FAPG Column back to the Collection Tube.
8. Repeat step 7 for the rest of the sample mixture.
9. Add 400 µl of Wash Buffer W1 (ethanol added) to the FAPG Column. Centrifuge at full speed (18,000 x g or 14,000 rpm) for 30 seconds. Discard the flow-through and place the FAPG Column back to the Collection Tube.
 - Make sure that ethanol (96~100%) has been added into Wash Buffer W1 when first use.
10. Add 650 µl of Wash Buffer W2 (ethanol added) to FAPG Column. Centrifuge at full speed (18,000 x g or 14,000 rpm) for 30 seconds. Discard the flow-through and place the FAPG Column back to the Collection Tube.
 - Make sure that ethanol (96~100%) has been added into Wash Buffer W2 when first use.
11. Repeat step 10 for one more washing.
12. Centrifuge at full speed (18,000 x g or 14,000 rpm) for an additional 3 min to dry the FAPG column completely.
 - Important step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
13. Combine the FAPG Column with a Elution Tube, Add 50~200 µl of preheated Elution Buffer to the membrane center of the FAPG Column. Stand the FAPG Column for 1 minute at room temperature.
 - Important step! For effective elution, make sure that the Elution Buffer is dispensed onto the membrane center and is absorbed completely.
14. Centrifuge at full speed (18,000 x g or 14,000 rpm) for 1 min to elute purified DNA.

Troubleshooting

Problems	Possible reasons	Solutions
Low or no yield of genomic DNA		
	Incorrect preparation of FAPG3 Buffer or Wash Buffer	
	FAPG3 Buffer is not mixed with ethanol before use	Repeat the extraction procedure with a new sample.
	Wash Buffer W1 and Wash Buffer W2 is not mixed with ethanol before use	Make sure that the correct volumes of ethanol (96-100 %) is added into Wash Buffer W1 and Wash Buffer W2 when first open. Repeat the extraction procedure with a new sample.
	The volume or the percentage of ethanol is not correct before adding into Wash Buffer W1 and Wash Buffer W2	Make sure that the correct volumes of ethanol (96-100 %) is added into Wash Buffer W1 and Wash Buffer W2 when first use. Repeat the extraction procedure with a new sample.
	Elution of genomic DNA is not efficient	
	pH of water (ddH ₂ O) for elution is acidic	Make sure the pH of ddH ₂ O is between 7.5- 9.0.
	Elution Buffer or ddH ₂ O is not completely absorbed by column membrane	Use Elution Buffer (provided) for elution. After Elution Buffer or ddH ₂ O is added, stand the PGDE Column for 5 min before centrifugation.
Column is clogged		
	Sample is too viscous	Reduce the sample volume.
Degradation of eluted DNA		
	Sample is old	Always use fresh or well-stored sample for genomic DNA extraction.
	Buffer for gel electrophoresis contaminated with DNase	Use fresh running buffer for gel electrophoresis.