

Kit Contents:

	FAPGK 000-Maxi (2 preps)	FAPGK 002 (10 preps)	FAPGK 002-1 (24 preps)
FAPG1 Buffer	10 ml	45 ml	110 ml
FAPG2 Buffer	3 ml	13 ml	30 ml
FAPG3 Buffer * (Concentrate)	7.5 ml	30 ml	70 ml
W1 Buffer * (Concentrate)	4.0 ml	26 ml	52 ml
Wash Buffer * (Concentrate)	5.0 ml	25 ml	50 ml
Elution Buffer	6 ml	30 ml	60 ml
RNase A Solution	50 µl	170 µl	170 µl × 2
Filter Column	2 pcs	10 pcs	24 pcs
FAPG Maxi Column	2 pcs	10 pcs	24 pcs
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* Preparation of FAPG1 Buffer and Wash Buffer at the first use:

Cat. No:	FAPGK000-Maxi (2 preps)	FAPGK002 (10 preps)	FAPGK002-1 (24 preps)
Ethanol volume for FAPG3 Buffer	15 ml	60 ml	140 ml
Ethanol volume for W1 Buffer	5 ml	34 ml	68 ml
Ethanol volume for Wash Buffer	20 ml	100 ml	200 ml

Specification:

Principle: spin column-maxi (silica membrane)

Sample: up to 1 g

Column Binding Capacity: ≤500 µg DNA/column

Operation time: <60 mins

DNA Yield: 50~300 µg

Important Notes:

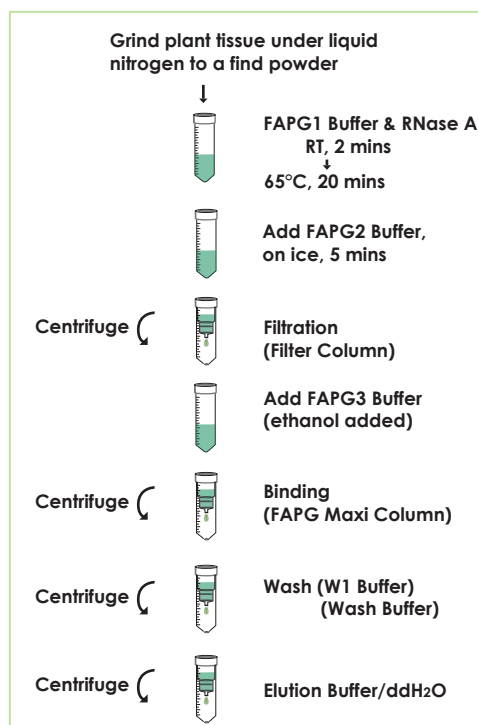
1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check FAPG1 Buffer before use (warm FAPG1 Buffer at 60°C for 5 mins if any precipitate form).
3. Preheat dry baths or water baths to 65°C before the operation.
4. Add required ethanol (96~100%) to FAPG3 Buffer, W1 Buffer and Wash Buffer before use.
5. Using a appropriate swing-bucket to centrifuge for 50 ml tube capable of speed 4,000~4,500 xg.
6. **Store RNase A Solution at -20°C upon receipt of kit.**

General Protocol:

HINT: Prepare a ice box and a 65°C bath for step 2 and 3.

Preheat Elution Buffer or ddH₂O to 65°C for step 11 (elution step).

1. **Cut off up to 1 g fresh or frozen plant tissue or 50 mg (up to 100 mg) dry plant tissue. Grind the sample under liquid nitrogen to a fine powder and transfer to a 15 ml centrifuge tube (not provided).**
 -For some plant sample, we can grind it without liquid nitrogen.
 -Do not allow the sample to thaw, and continue immediately to step 2.
2. **Add 4 ml of FAPG1 Buffer and 10 µl of RNase A Solution to the tissue powder. Vortex vigorously and incubate the mixture at room temperature for 2 mins and at 65°C for 20 mins, invert the tube 2~3 times during 65°C incubation.**
3. **Add 1 ml of FAPG2 Buffer to the mixture. Vortex to mix well and incubate the mixture on ice for 5 mins.**
4. **Place a Filter Column to a 50 ml tube and transfer the entire mixture from previous step to the Filter Column. Centrifuge the Filter Column with a swing-bucket rotor at speed (4,000~4,500 xg) for 5 mins.**
5. **Transfer the clarified lysate (supernatant) from the 50 ml tube to a new 50 ml tube (not provided). Adjust the volume of the 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 vortexing for 10 secs.**
 -Make sure that ethanol (96~100%) has been added to FAPG3 Buffer at the first use.
 -For explame: add 7.5 ml of FAPG3 (ethanol added) to 5 ml of lysate.
7. **Place a FAPG Maxi Column to a new 50 ml tube and transfer the sample mixture from step 6 to the FAPG Maxi Column. Centrifuge the FAPG Maxi a swing-bucket rotor at speed (4,000~4,500 xg) for 3 mins. Discard the flow-through and place the FAPG Maxi Column back to the 50 ml tube.**
8. **Add 4 ml of W1 Buffer (ethanol added) to the FAPG Maxi Column. Centrifuge the FAPG Maxi Column with swing-bucket rotor at speed (4,000~4,500 xg) for 3 mins. Discard the flow-through and place the FAPG Maxi Column back to the 50 ml tube.**
 -Make sure that ethanol (96~100%) has been added into W1 Buffe at the first open.
9. **Add 6 ml of Wash Buffer (ethanol added) to the FAPG Maxi Column. Centrifuge the FAPG Maxi Column with the swing-bucket rotor at speed at (4,000~4,500 xg) for 3 mins. Discard the flow-through and place the FAPG Maxi Column back to the 50 ml tube.**
 -Make sure that ethanol (96~100%) has been added into Wash Buffe at the first open.
10. **Centrifuge the FAPG Maxi Column with swing-bucket rotor at speed (4,000~4,500 xg) for an additional 10 mins to dry the FAPG Maxi Column completely.**
 -**Important step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
11. **Place the FAPG Maxi Column to a new 50 ml tube (not provided), Add 0.5~1 ml of preheated Elution Buffer or ddH₂O to the membrane center of the FAPG Maxi Column. Stand the FAPG Maxi Column for 5 mins at room temperature.**
 -**Important step!** For effective elution, make sure that the Elution Buffer or ddH₂O is dispensed onto the membrane center and is absorbed completely.
12. **Centrifuge the FAPG Maxi Column with a swing-bucket rotor at speed (4,000~4,500 xg) for 3 mins to elute the 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.
	W1 Buffer and Wash Buffer is not mixed with ethanol before use.	Make sure that the correct volumes of ethanol (96~100%) is added into W1 Buffer and Wash Buffer at the first open. Repeat the extraction procedure with a new sample.
	The volume or the percentage of ethanol is not correct before adding into W1 Buffer and Wash Buffer.	Make sure that the correct volumes of ethanol (96~100%) is added into W1 Buffer and Wash Buffer at the 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 FAPG Maxi Column for 5 mins before centrifugation.
Column is clogged.		
	Sample is too viscous.	Reduce the sample volume.
Degradation of elutated DNA.		
	Sample is old.	Always use fresh or well-conserved sample for genomic DNA extraction.
	Buffer for gel electrophoresis contaminated with DNase.	Use fresh running buffer for gel electrophoresis.