FavorPrep™ Plant Genomic DNA Extraction Maxi Kit

Cat.No. : FAPGK 000-Maxi
FAPGK 002
FAPGK 002-1

Kit Contents:

	FAPGK 000-Maxi (2 preps_sample)	FAPGK 002 (10 preps)	FAPGK 002-1 (24 preps)
FAPG1 Buffer	10 ml	45 ml	110 ml
FAPGX 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)	7.0 ml	33 ml	88 ml
Wash Buffer * (concentrate)	5.0 ml	25 ml	50 ml
Elution Buffer	6 ml	30 ml	60 ml
RNase A (lyophilized)	1.1 mg	5.5 mg	13 mg
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, W1 Buffer and Wash Buffer by adding 96 ~100% ethanol

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	2.5 ml	12 ml	32 ml
ethanol volume for Wash Buffer	20 ml	100 ml	200 ml

* Preparation of RNase A solution by adding Elution Buffer

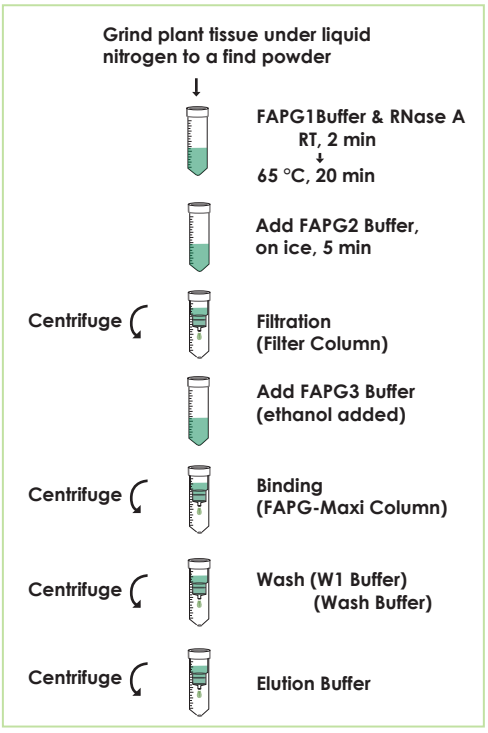
Cat. No:	FAPGK000-Maxi (2 preps)	FAPGK002 (10 preps)	FAPGK002-1 (24 preps)
Elution Buffer volume for RNase A tube	110 µl	550 µl	1.3 ml

Specification:

Principle: spin column - maxi (silica membrane)
Sample: up to 1 g
Operation time: < 60 min
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 minutes if any precipitate formd.
3. Preheat dry baths or water baths to 65˚C before the operation.
4. Add ethanol (96-100%) to FAPG3 Buffer, W1 Buffer and Wash Buffer before use. Store the ethanol added buffer at room temperature.
5. Add Elution Buffer to RNase A tube. Disslove well and store the solution at -20 °C before use.
6. Using a appropriate swing-bucket to centrifuge for 50 ml tube capable of speed 4,000 ~ 4.500 x g.



General Protocol:

HINT: Prepare a ice box and a 65 °C bath for step 2 and 3.
Preheat Elution Buffer or ddH_2O 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).**
   • Foe 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 (or FAPGX Buffer) and 50 µl of RNase A stock solution (10 mg/ml) to the tissue powder. Vortex vigorously and incubate the mixture at room temperature for 2 minutes and at 65°C for 20 minutes, invert the tube 2-3 times during 65 °C incubation.**
   • For some plant samples that contain a lot of polysaccharides, useing the FAPGX Buffer to lyse.
3. **Add 1 ml 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 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 x g) for 5 min.**
5. **Transfer the clarified lysate (supernatant) from the 50 ml tube to a new 50 ml tube (not provided). And adjust the volume of the clarified lysate.**
   • Note! Do not aspirate any debris when transfering the claarified lysate.

6. **Add 1.5 volume of FAPG3 Buffer (ethanol added) to the clarified lysate and mix well by vortexing for 10 seconds.**
   - Make sure that ethanol (96~100%) has been added to FAPG3 Buffer when 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 x g) for 3 min. 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 a swing-bucket rotor at speed (4,000 ~ 4,500 x g) for 3 min. 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 when first open.

9. **Add 6 ml of Wash Buffer (ethanol added) to the FAPG-Maxi Column. Centrifuge the FAPG-Maxi a swing-bucket rotor at speed (4,000 ~ 4,500 x g) for 3 min. 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 when first open.

10. **Centrifuge the FAPG-Maxi a swing-bucket rotor at speed (4,000 ~ 4,500 x g) for an additional 10 min 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 1 ml of preheated Elution Buffer or ddH$_2$O to the membrane center of the FAPG-Maxi Column. Stand the FAPG-Maxi Column for 5 minute at room temperature.**
    --Important step! For effective elution, make sure that the Elution Buffer or ddH$_2$O is dispensed onto the membrane center and is absorbed completely.

12. **Centrifuge the FAPG-Maxi with a swing-bucket rotor at speed (4,000 ~ 4,500 x g) for 3 min to elute thr 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 when 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 when first use. Repeat the extraction procedure with a new sample.
	Elution of genomic DNA is not efficient	
	pH of water (ddH2O) for elution is acidic	Make sure the pH of ddH2O is between 7.5- 9.0.
		Use Elution Buffer (provided) for elution.
	Elution Buffer or ddH2O is not completely absorbed by column membrane	After Elution Buffer or ddH2O is added, stand the PGDE-Maxi Column for 5 min 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-stored sample for genomic DNA extraction.
	Buffer for gel electrophoresis contaminated with DNase	Use fresh running buffer for gel electrophoresis.