α^+ Solution™
Plant Genomic DNA Extraction Mini Kit

Kit Contents:

Cat. No:	HBPGK 004 (4 preps_sample)	HBPGK 050 (50 preps)	HBPGK 100 (100 preps)
PG1 Buffer	2.0 ml	25 ml	55 ml
PG2 Buffer	1.0 ml	8 ml	15 ml
PG3 Buffer (concentrate)[a]	1.5 ml	15 ml	30 ml
GW Buffer (concentrate)[b]	0.8 ml	13 ml	26 ml
Wash Buffer (concentrate)[c]	1.5 ml	15 ml	30 ml
Elution Buffer	1.5 ml	15 ml	30 ml
RNase A (lyophilized)	1.5 mg	22 mg	43 mg
Filter Column	4 pcs	50 pcs	100 pcs
PG Column	4 pcs	50 pcs	100 pcs
Collection Tube	8 pcs	100 pcs	200 pcs
User Manual	1	1	1
Preparation of PG1 Buffer, GW Buffer and Wash for first use:			
Ethanol for PG3 Buffer [a]	3 ml	30 ml	60 ml
Ethanol for GW Buffer [b]	1.0 ml	17 ml	34 ml
Ethanol for Wash Buffer [c]	6 ml	60 ml	120 ml

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1. Wet weight ≤ 100 mg 2. Dry weight ≤ 20 mg
Operation time:	30 ~ 60 minutes
Binding capacity:	up to 60 µg total DNA / column
Expected yield:	5 ~ 40 µg / prep
Column applicability:	centrifugation and vaccum
Minimum elution volume:	50 µl

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check PG1 Buffer before use, Warm PG1 Buffer at 60℃ for 5 min if any precipitate formd.
3. Preheat dry baths or water baths to 65℃ before the operation.
4. **Add required ethanol (96-100%) to PG3 Buffer**, GW and Wash before use.
5. Store RNase A at -20℃ upon recipit of kit. Add sterile ddH_2O 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℃ .
6. All centrifuge steps are done at full speed(~ 18,000 x g) in a microcentrifuge.

Brief procedure:

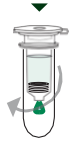
Sample Preparation

Grind 50 mg of wet weight (up to 100 mg) plant tissue or 20 mg dry weight of plant tissue with liquid nitrogen.

Lysis

1. **Add 400 µl of PG1 Buffer.**
2. **Add 8 µl of RNase A stock solution (50 mg/ml).**
3. Incubate at room temperature for 2 minutes then at 65 ℃ for 10~20 minutes.
4. **Add 130 µl of PG2 Buffer.**
5. Incubateon ice for 5 min.

Filtration



1. Transfer the entire mixture to the Filter Column.
2. Centrifugeat full speed (~ 18,000 x g) for 3 min.
3. Transfer the clarified lysate to a new microcentrifuge tube (not provided).
4. **Add 1.5 volume of PG3 Buffer (ethanol added).**

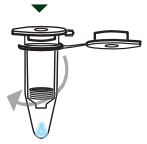
Binding

1. Transfer up to 750 µl of the sample mixture to PG mini column.
2. Centrifuge at at full speed (18,000 x g) for 1 min.
3. Repeat this step for the rest of the sample mixture.

Wash

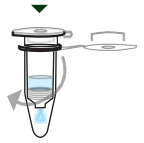
1. **Add 400 µl of GW Buffer.** Centrifuge at full speed (18,000 x g) for 30 seconds
2. **Add 650 µl of Wash Buffer** Centrifuge at full speed (18,000 x g) for 30 seconds. Repeat this step for one more washing.

Dry



Centrifuge at full speed (~18,000 x g) for an additional 3 min to dry the column.

Elution



Pure DNA

Add 50~200 µl of preheated Elution Buffer.
Centrifuge at full speed (18,000 x g) for 1 min.

General Protocol

Please Read Important Notes Before Starting Following Steps.

STEP	PROCEDURE
1 Sample preparation	Grind 50 mg of wet weight (up to 100 mg) plant tissue or 20 mg dry weight of plant tissue with liquid nitrogen to a fine powder and transfer to a new microcentrifuge tube (not provided).
2.1 Lysis	1. **Add 400 µl of PG1 Buffer** and **8 µl of RNase A stock solution (50 mg/ml)** to the tissue powder and vortex vigorously. 2. Incubate the mixture at room temperature for 2 minutes then at 65℃ for 10~20 minutes and invert 2-3 times during incubation.
2.2 Lysis	**Add 130 µl of PG2 Buffer** to the mixture. Vortex to mix well and incubate the mixture on ice for 5 min.
3 Filtration	1. 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. 2. Transfer the clarified lysate (supernatant) from the Collection Tube to a new microcentrifuge tube (not provided). 3. Discard used Filter Column and Collection Tube. And adjust the volume of clarified lysate. 4. **Add 1.5 volume of PG3 Buffer (ethanol added)** to the clarified lysate and mix well by pipetting.
4 DNA Binding	1. Place a PG Column to a new Collection Tube and transfer up to 750 µl of the sample mixture from previous step carefully to the PG Column. 2. Centrifuge at at full speed (18,000 x g) for 1 min. Discard the flow-through and place the PG Column back to the Collection Tube. 3. Repeat this step for the rest of the sample mixture.
5.1 Wash	1. **Add 400 µl of GW Buffer (ethanol added)** to the PG Column. 2. Centrifuge at full speed (18,000 x g) for 30 seconds than discard the flow-through.
5.2 Wash	1. **Add 650 µl of Wash Buffer (ethanol added)** to PG Column. Centrifuge at full speed (18,000 x g) for 30 seconds than discard the flow-through. 2. Repeat this step for one more washing.
6 Dry column	Centrifuge the PG Mini Column at full speed for an additional 3 min to dry the PG Mini Column.
7 DNA Elution	1. **Add 100 µl of preheated Elution Buffer or ddH_2O (pH 7.5-9.0)** to the membrane of the PG Mini Column. Stand the PG Mini Column for 3 min. 2. Centrifuge at full speed for 2 min to elute DNA.