Plant Genomic DNA Purification Kit

Cat # : PT-PGDNA Size : **50** Reactions

Components of the kit :

	50 reaction	**notice**
*Solution E1	19 ml	Precipitate can be easily dissolved by incubating at 50°C.
*Solution E2 powder	0.23 g x 2	Each tube add **1 ml** sterile water to dissolve. **Store the solution E2 at −20°C.**
* RNase A	10 mg	**Store at 4°C when received the kit.** Add 200 ul sterile water before use.
Solution P1	7.5 ml	
* Binding Buffer	12.5 ml	Add **25 ml Ethanol** before using
* Wash buffer	12.5 ml	Add **50 ml Ethanol** before using
Elution Buffer	7.5 ml	Preheated the solution at 60-70
Filter column & collection tube	50 sets	
Binding column & collection tube	50 sets	

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Special Note:

Sample size and yield:
Up to 100 mg for wet leaf weight, 50 mg for fungi.
Typical yield: 1-20 ug

Materials to be supplied by the user:
For tissue grinding: mortar and pestle.
Liquid nitrogen
Ethanol

Before starting:
Thaw the solution E2 before grinding tissue.
Preheat water bath or heating block at 65°C.

Step	Procedures
Sample Preparation	**1.** Add liquid nitrogen and plant tissue (<100 mg) in mortar and pestle, grind the sample into fine powder. **Do not let sample to thaw.** **2.** Immediately transfer the powder to 1.5 ml tube, add 360 µl Solution E1 , 40µl Solution E2 and 4 µl RNaseA Solution. Vortex vigorously for 5-10 sec.
Cell Lysis and removing proteins, polysaccharides	**3.** Incubating at 65°C in water-bath or heating block for 20 min. Mix by inverting the tube from time to time. **4.** Add 130µl Solution P1, mix by inverting the tube, and incubate on ice for 5 min. **5.** Centrifuge at top speed (12-14,000 rpm) for 5 min at RT. **6.** Collect the supernatant into **Filter Column** sitting in a clean 2 ml **collection tube**. Spin at top speed for 2 min. **7.** Collect the flow-through buffer in the collection tube into a new 1.5 ml tube, avoid pipetting the pellets.
Binding DNA	**8.** Add 700µl of Binding Buffer and mix thoroughly by pipetting. **9.** Transfer 650 µl of the mixture including any precipitate into **Binding Column** and spin for 1 min at top speed and discard the flow-through. **10.** Repeat step 9 with remaining mixture.
Wash DNA	**11.** Discard the flow-through, wash by adding 600 µl of Wash Buffer to the column, then spin at top speed (12-14,000 rpm) for 1 min. **12.** Discard the flow-through and repeat this wash step. **13.** Discard the flow-through, then centrifuge for 5 min at top speed (12-14,000 rpm) to remove ethanol.
Eluting DNA	**14.** Discard the collection tube and place the column into a new 1.5 ml centrifuge tube. **15.** Add preheated 60-70 of 50-100 µl **Elution Buffer or H_2O (pH should be between 7.0-8.5)** into **centre** of the column, **wait for 2 min.** **16.** Eluting the DNA by centrifugation for 2 min, discard the column and store the DNA at -20 . ** Repeat elution once by using the same eluate and the same column may have 10-15% more DNA yield.*

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