HiPure HP Plant DNA Kit

Introduction

HiPure HP Plant DNA Kit supplies a simple and rapid extraction of genomic DNA from different plant samples. The kit is based on silica gel column and CTAB lysis purification technology. The whole extraction process is only 30~50 minutes. Purified DNA can be used directly for PCR, SSR, AFLP, RAPD and Southern Blot, ect.

Principle

This product is based on silica column purification. The sample is lysed with CATB Buffer, DNA is released into the lysate. Cell debris, precipitated proteins and polysaccharides are removed by chloroform extraction. After adjust the binding condition, transfer to an adsorption column. DNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, Nucleic acid was finally eluted with low-salt buffer (10mm Tris,pH9.0, 0.5mm EDTA).

Kit Contents

Cat.No.	D318700	D318702	D318703
Purification times	20 Preps	50 Preps	250 Preps
HiPure DNA Mini Columns II	20	50	250
2ml Collectoin Tubes	20	50	250
Buffer PAL	15 ml	60 ml	200 ml
Buffer GWP	15 ml	60 ml	200 ml
Buffer DW1	15 ml	30 ml	150 ml
Buffer GW2*	6 ml	20 ml	50 ml
Buffer AE	5 ml	20 ml	60 ml

Storage and Stability

This product can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

Materials and Equipment to be Supplied by User

- Heat block or water bath capable of 65℃
- 100% ethanol
- Chloroform
- (Optional) 2-mercaptoethanol and PVP-40
- Add 24ml (20 Preps) or 80ml (50 Preps) or 200ml (250 Preps) 100% ethanol to the bottle of Buffer GW2 and store at room temperature.

Protocol A

1. **Grind plant tissue/fungus sample into powder by liquid nitrogen. Transfer 50~150mg fresh/frozen sample or 15~40mg dry sample into a 2ml centrifuge tube.**
   Ideal results is based on suitable sample amount in use. Too much sample will decrease DNA yield and purity because of column overload and inefficient lysis. For the first time, we recommend to use 50mg fresh or 15mg dry sample as different plant samples have big difference on DNA amount. And adjust sample amount according to the test result. For mucus rich sample, we recommend to use on 30~50mg sample (fresh) reach time.

2. **Add 700µl Buffer PAL (pre-heated to 65℃) to the sample immediately**. Vortex vigorously to disperse the samples. Incubate at 65℃ for 20 minutes and Invert to mix 2~3 times.
   For treating complex samples, add 2-mercaptoethanol to Buffer PAL in a 0.1% (v/v) concentration to improve the oxidation resistance of the lysis solution. For extremely hard lysis sample, can add 2-mercaptoethanol to Buffer PAL in a 2% (v/v) concentration. For simple crash crops such as rice, corn, tomato, addition of 2-mercaptoethanol is optional.

3. **Add 700µl chloroform to the sample and vortex for 15 seconds.**
   For treating polyphenol or starch rich sample, add phenol:chloroform/1:1 (v:v) extraction step before step 3.

4. **Centrifuge at 12,000 x g for 5 minutes at room temperature.** Transfer the cleared supernatant to a new centrifuge tube carefully.
   If RNA-free genomic DNA is required, add 10µl RNase A (25mg/ml) and incubate for 15min at room temperature.

5. **Add 700µl Buffer GWP to the supernatant, and overturn the tube 10~15 times to mix.**

6. Insert a HiPure gDNA Mini Column II into a 2.0mL Collection Tube (provided).

7. **Pipet 750 µl of the mixture from step 7, including any precipitate that may have formed, into the column placed in a 2 ml collection tube (supplied).** Centrifuge for 1 min at ⩾10000 x *g* and discard the flow-through. Reuse the collection tube in step 10.

8. Repeat step 7 with remaining sample. Discard flow-through and Reuse the collection tube.

9. Discard the filtrate and reuse collection tube. **Add 500μl Buffer DW1 to the column.** Centrifuge at 12,000 x g for minute.

10. Discard the filtrate and reuse collection tube. **Add 500μl Buffer GW2 to the column.** Centrifuge at 12,000 x g for minute.

11. (for choice) repeat Step 10.

12. Discard the filtrate and reuse the collection tube. Centrifuge the empty column at 12, 000 x g for 2 minutes to dry the column.

13. **Place the column to a clean 1.5ml centrifuge tube. Add 40-75μl Buffer AE (preheated to 65℃) directly to the center of the column membrane.** Let sit at room temperature for 2 minutes. Centrifuge at 12,000 x g for 1 minute.

14. **Add 40-75μl Buffer AE (preheated to 65℃) directly to the center of the column membrane.** Let sit at room temperature for 2 minutes. Centrifuge at 12,000 x g for 1 minute

15. Discard the column and store DNA at 2~8℃. For long time storage, store at -20℃.

Protocol B

This protocol is used for polyphenol-rich plant or fungal samples. Add PVP-40 to Buffer PAL at a 2% concentration (W/V), mix throughly to mix well. This mixture should not stay at room temperature above 1 week. Then add 2-mercaptoethanol to Buffer PAL at a 1% concentration (V/V) before use.

1. **Grind plant tissue/fungus sample into powder by liquid nitrogen. Transfer 50~100mg fresh/frozen sample or 15~30mg dry sample into a 2ml centrifuge tube.**
   Ideal results is based on suitable sample amount in use. Too much sample will decrease DNA yield and purity because of column overload and inefficient lysis. For the first time, we recommend to use 50mg fresh or 15mg dry sample as different plant samples have big difference on DNA amount. And adjust sample amount according to the test result.

2. **Add 700μl Buffer PAL/PVP-40 (pre-heated to 65℃) to the sample immediately**. Vortex fiercely to disperse the samples throughly. Incubate at 65℃ for 15~30 minutes and overturn the tube to mix 2~3 times.

3. **Add 700μl chloroform to the sample and vortex for 15 seconds.** Centrifuge at 12,000 x g for 5 minutes
   For treating polyphenol or starch rich sample, add phenol:chloroform/1:1 (v:v) extraction step before step 3.

4. Transfer 600μl cleared supernatant to a new centrifuge tube carefully.
If RNA-free genomic DNA is required, add 10μl RNase A (25mg/ml) and incubate for 15min at room temperature.

5. **Add 300μl Buffer GWP and 600μl 100% ethanol to supernatant, and overturn the tube 10~15 times to mix thoroughly.** If sediment pellet appears, use pipette pipetting up and down to disperse the pellet. Follow Step 6~15 in Protocol A.

Troubleshooting Guide

1. Clogged DNeasy membrane

- **Lysate too viscous:** Reduce the amount of starting material and/or increase the amounts of Buffer PAL.

2. Low or no recovery

- **Buffer GW2 did not contain ethanol:** Ethanol must be added to Buffer GW2 before used. Repeat procedure with correctly prepare Buffer.
- **Insufficient disruption:** Ensure that the starting material is completely disrupted.
- **Insufficient lysis:** Reduce the amount of starting material and/or increase the amounts of Buffer PAL.

3. Darkly colored membrane or green/yellow eluate after washing with Buffer GW2

- Too much starting material Reduce the amount of starting material in future preps.
- Insufficient washing of the membrane: After washing with Buffer GW2 (step 10), perform an additional wash with 500 μl ethanol (96–100%). Centrifuge for 2 min at 20,000 x g (14,000 rpm) to dry the membrane.

4. DNA does not perform well (e.g. in ligation reaction)

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 5 min at room temperature after adding 500ul of Buffer GW2, then centriufge or Vacuum.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >12,000 x g for 1min, then dry.
- **Inappropriate elution volume used:** Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.