【Product Name】HiPure HP Plant DNA Maxi Kit

【Product specifications】10 Preps/Kit, 50 Preps/Kit

【Intended Use】

This product provide a fast and easy way to purify DNA from plant and fungal tissue. Up to 3 g of tissue can be processed.Easy-to-use Plant procedures provide pure total DNA (genomic, mitochondrial, and chloroplast) for reliable PCR and Southern blotting in less than 1 hour.

【Principle】

This product is based on silica Column purification. Plant material is first mechanically disrupted and then lysed by addition of lysis buffer and incubation. RNase A in the lysis buffer digests the RNA in the sample. After lysis, proteins and polysaccharides are removed by chloroform. Binding buffer and ethanol are added to the cleared lysate to promote binding of the DNA to the HiPure membrane. The sample is then applied to a column and then centrifuged. DNA binds to the membrane, while contaminants such as proteins and polysaccharides are efficiently removed by 2 wash steps. Pure DNA is eluted in a small volume of low-salt buffer or water.

【Kit Contents】

Cat.No.	D316302	D316303	Main Composition
Purification Times	10	50	-
HiPure DNA Maxi Columns II	10	50	Silicon Column
50ml Collection Tubes	20	100	PP Column
RNase A	20 mg	90 mg	Ribonuclease
Protease Dissolve Buffer	1.8 ml	10 ml	Tris/Glycorel/CaCl2
Buffer PAL	180 ml	900 ml	Tris/EDTA/CTAB
Buffer GWP	150 ml	800 ml	Guanidine Salt
Buffer GW2*	25 ml	200 ml	Tris/NaCl
Buffer AE	30 ml	120 ml	Tris/EDTA

【Storage conditions and Validity】

RNase A should be stored at 2 – 8° C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15 – 25° C) does not affect its performance. The remaining kit components can be stored dry at room temperature (15 – 25° C) and are stable for at least 18 months under these conditions.The entire kit can be stored at 2 – 8° C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

【Preparation before Use】

- Add 100ml (10 Preps) or 800ml (50 Preps) absolute ethanol to each bottle of Buffer GW2 and store at room temperature.
- Chloroform or Chloroform-Isoamylol (24:1)
- Add 1.2ml (10Preps) or 6ml (50Preps) Protease Dissolve Buffer to the bottle of RNase A and store at -20-8°C.

【Protocol for Plant and Fungal Tissue】

1. **Disrupt plant or fungal tissue by Liquid nitrogen ground.**
   Plant or fungal tissue can be ground to a fine powder under liquid nitrogen using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw.
2. **Add 16ml Buffer PAL to a maximum of 3 g (wet weight) or 0.6 g (dried-weight) disrupted plant or fungal tissue and vortex vigorously.**
   Vortex or pipet further to remove any clumps. Clumps of tissue will not lyse properly and will therefore result in a lower yield of DNA. In rare cases, where clumps cannot be removed by pipetting and vortexing, a disposable micropestle may be used. Do not add PVP-40 to Buffer PAL in this protocol. 2-mercaptoethanol can be added to improve DNA yield in this protocol.
3. Incubate the mixture for 30min at 65°C. Mix 3-5 times during incubation by inverting tube.
4. **Add 16ml chloroform to the lysate. Mix well by vortex 15s.**
5. Centrifuge the lysate for 10 min at >4,000 x *g*.
6. **Transfer the 13ml of the supernatant into a new tube (not supplied) without disturbing the pellet.**
7. Add 100μl RNase A to the supernatant and mix well. Incubate at room temperature for 10 min.
8. **Add 13ml Buffer GWP to the cleared lysate, and mix by inverting the tube 6~8 times.**

For example, to 13ml supernatant, add 13ml Buffer GWP. Reduce the amount of Buffer GWP accordingly if the volume of supernatant is smaller.

9. Insert a HiPure DNA Maxi Column II into a 50mL Collection Tube (provided).

10. **Pipet one half of the mixture from step 8 into the column placed in a 50 ml collection tube (supplied).** Centrifuge at 3000–5000 x g for 5 min at room temperature in a swing-out rotor.

11. Repeat step 10 with remaining sample. Discard flow-through and Reuse the collection tube.

12. **Add 10 ml Buffer GW2 to the column and centrifuge for 3 min at 3,000-5,000 x *g* .** Discard the flow-through and reuse the collection tube in step 12.

13. **Add 10ml absolute ethanol to the column, and centrifuge for 3 min at 3,000-5,000 x *g*.**

14. Discard the flow through and reuse the collection Tubes.Centrifuge at 3000–5000 x g for 10 min at room temperature in a swing-out rotor.

15. **Transfer the column to a 50 ml centrifuge tube, and pipet 0.75~1.0 ml Buffer AE directly onto the membrane.** Incubate for 5 min at room temperature. Centrifuge at 3000–5000 x g for 5 min at room temperature in a swing-out rotor.
Elution may also be performed with 0.5ml of Buffer AE (instead of 0.75–1ml). This increases the final DNA concentration in the eluate, but also reduces overall DNA yield.

16. **Repeat step 15 once.** Store DNA at -20°C.

【Protocol for polyphenol-Rich Plant and Fungal Tissue】

1. **Disrupt plant or fungal tissue by Liquid nitrogen ground.**
Plant or fungal tissue can be ground to a fine powder under liquid nitrogen using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw.

2. **Add 13ml Buffer PAL/PVP-40 to a maximum of 2g (wet weight) or 500 mg (dried weight) disrupted plant or fungal tissue and vortex vigorously.**

   Add PVP-40 (Polyvinylpyrrolidone 40, no provided) and 2-mercaptoethanol (no provided) to Buffer PAL to final concentration of 2% before use. Vortex or pipet further to remove any clumps. Clumps of tissue will not lyse properly and will therefore result in a lower yield of DNA.

3. Incubate the mixture for 30 min at 65°C. Mix 3-5 times during incubation by inverting tube.

4. **Add 13ml chloform to the lysate. Mix well and incubate at room temperature for 3 min.** Centrifuge the lysate for 10 min at >3,000 x *g*.

5. **Transfer the 10ml of the supernatant into a new tube and add 100μl RNase A.** Mix well and incubate at room temperature for 20 min.

6. **Add 5ml GWP to the sample and invert the tube 4-6 times to mix well.**

7. **Add 10ml absolute ethaol and mix thoroughly by vortexing for 15s.**
A precipitate may form after the addition of ethanol, but this will not affect the procedure.

8. Flow step 9-16.

Troubleshooting Guide

1. Clogged DNeasy membrane

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

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 and chloroform.

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, perform an additional wash with 500 μl ethanol (96–100%).

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.