【Product Name】HiPure SF Plant DNA Kit

【Product Specifications】50 Preps/Kit, 250 Preps/Kits

【Intended Use】

This product provide a fast and easy way to purify DNA from plant and fungal tissue. Up to 100 mg 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. Purification requires no phenol or chloroform extraction or alcohol precipitation and involves minimal handling.

【Principle】

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 salt-precipitated. Binding buffer 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 3 wash steps. Pure DNA is eluted in a small volume of low-salt buffer or water.

【Kit Contents】

Cat.No.	D316402	D316403	Main Composition
Purification Times	50	250	-
HiPure gDNA Mini Columns	50	2 x 125	Silicon Column
2ml Collection Tubes	50	2 x 125	PP Column
Buffer SPL	30 ml	150 ml	Tris/EDTA/SDS
Buffer PS	10 ml	50 ml	KAc
Buffer GW1*	22 ml	2 x 110 ml	Guanidine Salt
Buffer GW2*	12 ml	2 x 50 ml	Tris/NaCl
RNase A	10 mg	50 mg	Ribonuclease
Protease Dissolve Buffer	1.8 ml	5 ml	Glycerol/Tris/CaCl2
Buffer AE	15 ml	60 ml	Tris/EDTA

【Storage conditions and Validity】

RNase A should be stored at 2–8°C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15–25°C) does not affect their 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.

【Preparation before Use】

- Add 48ml (50Preps) or 2 x 200ml (250 Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 28ml (50Preps) or 2 x 140ml (250 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 0.6ml (50Preps) or 3ml (250 Preps) Protease Dissolve Buffer to the RNase A and store at -20~8℃ after dissolve.

【Protocol for Plant and Fungal Tissue】

1. **Disrupt plant or fungal tissue by Liquid nitrogen ground or other bead-beat methods.**
   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 600µl Buffer SPL and 10µl RNase A to a maximum of 100 mg (wet weight) or 20 mg (dried) 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. In rare cases, where clumps cannot be removed by pipetting and vortexing, a disposable micropestle may be used. Do not mix Buffer SPL and RNase A before use.
3. Incubate the mixture for 10 min at 65°C. Mix 2–3 times during incubation by inverting tube.
4. **Add 200µl Buffer PS to the lysate. Mix by vortex and incubate for 5 min on ice.**
5. Centrifuge the lysate for 5 min at >13,000 x *g*.
6. **Transfer the 600µl of the supernatant into a new tube (not supplied) without disturbing the cell-debris pellet.**
7. **Add 1.5 volumes of Buffer GW1 to the cleared lysate, and mix by pipetting.**
   For example, to 600µl supernatant, add 900µl Buffer GW1. Reduce the amount of Buffer GW1 accordingly if the volume of supernatant is smaller. A precipitate may form after the addition of Buffer

GW1, but this will not affect the procedure. Ensure that ethanol has been added to Buffer GW1. It is important to pipet Buffer GW1 directly onto the supernatant and to mix immediately.

8. Insert a HiPure gDNA Mini Column II into a 2.0mL Collection Tube (provided).
9. **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.
10. Repeat step 9 with remaining sample. Discard flow-through and Reuse the collection tube.
11. **Add 500 µl Buffer GW1 and centrifuge for 1 min at ≥10000 x *g* .** Discard the flow-through and reuse the collection tube in step 12.
12. **Add 650 µl Buffer GW2 and centrifuge for 1 min at ≥10000 x *g* .** Discard the flow-through and reuse the collection tube in step 13.
13. **Add 650µl Buffer GW2 to the column, and centrifuge for 1 min at 10,000 x *g*.**
    After washing with Buffer GW2, the column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer GW2, perform an additional wash with 500 µl ethanol (96–100%). Centrifuge for 1 min at 10,000 x g.
14. Discard the flow through and reuse the collection Tubes. Centrifuge at 10,000 x g for 1 min. This step helps to eliminate the chance of possible Buffer GW2 carryover.
15. **Transfer the column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 50~100µl Buffer AE directly onto the membrane.** Incubate for 5 min at room temperature, and then centrifuge for 1 min at 10,000 x *g* to elute.
    If larger amounts of DNA (>20 µg) are loaded, eluting with 200 µl (instead of 100 µl) increases yield.
16. **Repeat step 15 once.** Store DNA at -20°C
    A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the microcentrifuge tube can be reused for the second elution step to combine the eluates.

Troubleshooting Guide

1. Clogged DNeasy membrane

- **Lysate too viscous:** Reduce the amount of starting material and/or increase the amounts of Buffer SPL and Buffer PS.
- **Insufficient centrifugation:** Increase the g-force and centrifugation time.

2. Low or no recovery

- **Buffer GW1/GW2 did not contain ethanol:** Ethanol must be added to Buffer GW1/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 SPL and Buffer PS.
- **Incorrect binding conditions:** Make sure that the amount of lysate is accurately determined so that the correct amount of Buffer GW1 is added to adjust binding conditions correctly

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 13), 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.