HiPure SF Plant DNA 96 Kit

Introduction

This product is suitable for rapid extraction of DNA from plant and fungal samples. The kit is based on silica gel column purification technology. The whole extraction process is only 60~90 minutes. DNA can be used directly for PCR, quantitative PCR, Southern Blot, test of virus DNA and so on.

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.	D316701	D316702	D316703
Purification times	1 x 96 Preps	4 x 96 Preps	20 x 96 Preps
HiPure gDNA Plate	1	4	20
2.2ml Collection Plate	2	8	40
1.6ml Collection Plate	1	4	20
0.8ml Collection Plate	1	4	20
Sealing Film	10	20	100
Buffer SPL	60 ml	200 ml	2 x 500 ml
Buffer PS	20 ml	100 ml	400 ml
Buffer GW1*	66 ml	220 ml	5 x 220 ml
Buffer GW2*	50 ml	100 ml	3 x 100 ml
RNase Solution	1.2 ml	5 ml	22 ml
Elution Buffer	30 ml	120 ml	500 ml

Storage and Stability

RNase Solution 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 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.

Materials and Equipment to be Supplied by User

- Add 200ml (D316701) or 400ml (D316702) or 3 x 400ml (D316703) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 84ml (D316701) or 280ml (D316702) or 5 x 280ml (D316703) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.

Protocol

1. **Transfer up to 15 mg dry powdered tissue or 50 mg fresh (or frozen) powdered tissue to a 2.2 mL Collection Plate.**
   No more than 50 mg (wet weight) or 15 mg (dry weight) starting material is recommended. More or less can be used depending on results. Water content (and buffer absorption) of samples affect optimal starting amounts.
2. **Add 400µL Buffer SPL and 10µL RNase A to each sample.** Seal the wells with sealing film (not Provided). Vortex at maxi speed for 30~90 seconds.
3. Incubate at 65°C for 10 minutes. Mix samples twice during incubation by briefly shaking the plate for 10~30 seconds.
4. Centrifuge briefly to collect any drops of liquid from the mat.
5. **Remove and discard the sealing film, Add 130µL Buffer PS to each lysate.** Seal the wells with sealing film. Vortex at maxi speed for 30~60 seconds. Incubate at -20°C for 10 minutes.
6. Centrifuge at 4,000-5,000 x g for 15 minutes.
7. **Remove and discard the sealing film. Transfer 400µL supernatant to 2.2ml Collection Plate.**
8. **Add 1.5 volumes Buffer GW1 to the sample and seal the wells with sealing film.** Vortex at 700~900rpm for 60~90seconds. A precipitate may form at this point; it will not interfere with DNA isolation.

Spin Procedure

9. Insert a HiPure DNA Plate onto a 1.6mL Collection Tube (provided).

10. **Pipet 500µl of the mixture from step 8 into the plate placed in a 1.6ml collection plate (supplied).** Centrifuge for 3 min at >4,000 x *g* and discard the flow-through. Reuse collection plate in step 11.

11. Repeat step 10 with remaining sample. Discard flow-through and reuse the collection plate.

12. **Add 500µl Buffer GW1 and incbuate for 1 min. Centrifuge for 3 min at 4,000 x *g* .** Discard the flow-through and reuse the collection plate in step 13.

13. **Add 700µl Buffer GW2 to each well of plate and centrifuge for 3 min at >4,000 x *g* .** Discard the flow-through and reuse the collection tube in step 14.

14. **Add 650µl Absolute ethanol to each well of the plate, and centrifuge for 3 min at >4,000 x *g*.** Discard the flow-through and reuse the collection tube in step 15.

15. Centrifuge at >4,000 x g for 10 min.

16. Allow to air dry for 10 min at room temperature.

17. **Transfer the plate onto a 0.8ml Collection tube, and pipet 75~100µl Buffer AE directly onto the membrane.** Incubate for 5 min at room temperature, and then centrifuge for 3 min at >4,000 x *g* to elute.
    Important: Ensure that the buffer AE is dispensed directly onto the center of HiPure membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume. A second elution step with a further 75-100µl Buffer AE increases yields by up to 20~30%.

Vacuum Procedure:

9. Prepare the vacuum manifold according to manufacturer's instructions.

10. Place an HiPure gDNA Plate on the top part of the vacuum manifold.

11. **Transfer 1mL sample (including any precipitate that may have formed) from Step 8 to the HiPure gDNA Plate.**

12. Turn on the vacuum source to draw the sample through the plate. Turn off the vacuum.

13. **Add 500µL Buffer GW1 to each well.** Turn on the vacuum source to draw the Buffer GW1 through the plate. Turn off the vacuum.

14. **Add 700µL Buffer GW2 to each well.** Turn on the vacuum source to draw the Buffer GW2 through the plate. Turn off the vacuum.

15. **Add 700µL Absolute ethanol to each well.** Turn on the vacuum source to draw through the plate. After Absolute ethanol in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.

This step removes residual ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

16. Switch off vacuum source. **Vigorously tap the HiPure DNA plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the plate with clean absorbent paper.**
This step removes residual Absoluet ethanol from around the outlet nozzles and collars of the k plate. Residual ethanol may inhibit subsequent enzymatic reactions, e.g., sequencing.

17. **Transfer the HiPure DNA Plate into a new 0.8ml Collection Plate. Add 75~100µl Buffer AE to the center of the membrane. let the column stand for 2 min.** Centrifuge at 4,000 × g for 3 minute at room temperature.
Ensure that the buffer AE is dispensed directly onto the center of HiPure membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume. A second elution step with a further 75-100µl Buffer AE increases yields by up to 20~30%.

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. 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.