MagPure Plant DNA Kit

Introduction

This Kit supplies a simple and rapid extraction of genomic DNA from different plant pieces and tissues. The kit is based on superparamagnetic particles purification technology, no phenol-chloroform extraction or alcohol precipitation. The whole extraction process is only 60 minutes. This kit can use on different automatic extraction machines like KingFisher ML, KingFisher Flex and KingFisher Duo. Purified DNA can be used directly for PCR, quantitative PCR, Southern Blot, hybridization, and transgenosis detection.

Principle

This product is based on the purification method of high binding magnetic particles.Samples are first mechanically disrupted and then chemically lysed. RNA is removed by RNase digestion during lysis. Cell debris, precipitated proteins and polysaccharides are removed by add high salt solution. After adding magnetic particles and binding solution, DNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing buffer to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA was eluted by Elution Buffer.

Kit Contents

Cat.No.	D635100	D635101	D635102	D635103
Purification Times	20 Preps	48 Preps	96 Preps	5 x 96 Preps
MagPure Particles	0.8 ml	1.7 ml	4 ml	18 ml
RNase Solution	0.3 ml	0.6 ml	1.2 ml	6 ml
Buffer SPL	15 ml	30 ml	60 ml	300 ml
Buffer PS	5 ml	10 ml	20 ml	100 ml
Buffer GW1*	13 ml	26 ml	53 ml	220 ml
Elution Buffer	5 ml	10 ml	30 ml	120 ml

Storage and Stability

RNase Solution and MagPure Particles 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.

Materials and Equipment to be Supplied by User

- 80% ethanol and 95~100% ethanol
- Magnetic Particles should be shake violently for 1 minutes to be homogeneous
- Add 17ml (20Preps), 34ml (48 Preps) or 67ml (96 Preps) or 280ml (5 x 96 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature
- Heat block or water bath capable of 65℃
- **Add 2-mercaptoethanol:** when dealing with complicated samples, add 2-mercaptoethanol to Buffer SPL (2%) will improve the antioxidant capacity of lysate. For example, add 20μl 2-mercaptoethanol to 1ml Buffer SPL.
- **Complicated sample:** This kit has good performance on common commercial crops. For complicated samples like woody plants, users can optimize the process according to the sample type. When optimizing the process, we recommend to adjust the sample amount first. Secondly, ensure 2-mercaptoethanol is added to the Buffer SPL to improve the antioxidant capacity of the lysate. Finally, use CTAB lysis/chloroform extraction instead of Buffer SPL/PS extraction process. Drying or polysaccharide plant samples, lysis with CTAB buffer and extraction with chloroform can more effectively remove polysaccharide impurities.

Protocol :

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 500μl Buffer SPL and 10μl RNase Solution 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 Solution before use.

3. Incubate the mixture for 10 min at 65°C. Mix 2–3 times during incubation by inverting tube.

4. **Add 170µl Buffer PS to the lysate. Mix by vortex and incubate for 5 min on ice.** Centrifuge the lysate for 5 min at >13,000 x *g*.

Manual Purify:

5. **Transfer 500µl of the supernatant (step 5) into a new 1.5ml centrifuge tube (not supplied) without disturbing the cell-debris pellet.**

6. **Add 30µl MagPure Particles and 350µl 100% ethanol to the sample tube.** Pipette mix 20 times or shaking for 5 minutes.

   The liquid should appear homogeneous after mixing. Shaking samples for 5 min, instead of pipette mixing, may result in higher yield.

7. Place the tube to the magnetic stand for 2 minutes, until the MagPure Particles have formed a tight pellet, then remove the supernatant.

7. **Add 500µl Buffer GW1, resuspend the beads by shaking for 1min. .** Place the tube to the magnetic stand for 1 minute, then remove the supernatant.

8. **Add 500µl Buffer GW1, resuspend the beads by shaking for 1min. .** Place the tube to the magnetic stand for 1 minute, then remove the supernatant.

9. **Add 600µl 80% ethanol , resuspend the beads by shaking for 1min.** Place the tube to the magnetic stand for 1 minute, then remove the supernatant.

10. **Add 600µl absolute ethanol , resuspend the beads by shaking for 1min.** Place the tube to the magnetic stand for 1 minute, then remove the supernatant.

11. Dry on air for 10 minutes.

12. **Add 50-100µl Elution Buffer to the sample,** suspend the particles by vortex. Incubate at 65°C for 10 minutes by shaking. If there is no shaking device, vortex 2~3 times to mix DNA with magnetic particles. Place the tube to the magnetic rack for 2 minutes.

13. Transfer the supernatant containing the purified DNA to a clean 1.5ml centrifuge tube.

Auto Pure by KingFisher Flex Protocol

1. Add the Reagents/sample to the well of f the deep well plate according to the table below.

Name of the Plate	Pre-loaded reagents	Addition before use
Sample plate	350µl absolute ethanol 500µl Superntant from step 4.	
Wash Plate 1	500µl Buffer GW1, Put in 96 magnetic Tip 30µl MagPure Particles	
Wash Plate 2	500µl Buffer GW1	
Wash Plate 3	750µl 80% Ethanol	
Wash Plate 4	750µl absolute ethanol	
Elution plate	100µl Elution Buffer	

2. Place a 96 tip comb for deep well magnets on Wash Plate 1.
3. Start the protocol with the KingFisher Flex and load the plates.
4. After the run is completed, remove the plates and store the purified total DNA.

Troubleshooting Guide

1. Low or no recovery

- **Buffer GW1 did not contain ethanol:** Ethanol must be added to Buffer GW1before 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 absolute ethanol is added to adjust binding conditions correctly

2. DNA does not perform well

- **MagPure particles not dispersed throughly** : Shake the MagPure Particles violently to be homogeneous before use.
- **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.