MagPure Seed DNA Kit

Introduction

MagPure Seed DNA Kit supplies a simple and rapid extraction of genomic DNA from different plant pieces and seed. 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. 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.	D635201	D635202	D635203
Purification times	48 Preps	96 Preps	5 x 96 Preps
MagPure Particles	1.7 ml	4 ml	18 ml
Buffer SOL	40 ml	90 ml	500 ml
Buffer SDS	4 ml	9 ml	50 ml
Buffer MPB	40 ml	70 ml	350 ml
Buffer GW1*	26 ml	53 ml	220 ml
Elution Buffer	10 ml	30 ml	120 ml

Storage and Stability

MagPure Particles should be stored at 2–8°C upon arrival. However, short-term storage (up to 24weeks) 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
- MagPure Particles should be shake violently for 1 minutes to be homogeneous
- Magnetic separating device for single well or 96 well deep-well plates
- Heat block or water bath capable of 65℃
- Add 34ml (48 Preps), 67ml (96 Preps) or 280ml (5 x 96 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.

Protocol :

Plant Seed (high yield):

1. The Plant seeds are ground into fine powder by high speed grinder and transfer 30-100 mg of the powders to 2ml microcentrifuge tube.
2. **Add one 3 mm tungsten carbide bead to the tube and add 0.8ml Buffer SOL to the sample.** Vortex at high speed for 10 min or Place the tubes into tissuelyser or GeneGrinder. Homogenize the samples for 3~5 min at 30~50 Hz. Proceed step 3.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x *g*.

Fast Protocol（young leaf/fruit):

1. **Place a tender sample (young leaf or fruit) into a 2ml safe-lock microcentrifuge tube containing beads (one 3mm tungsten).** If processing fresh or frozen plant tissue, 50 mg of starting material is sufficient.
2. **Add 0.7ml Buffer SOL to the sample and place the tubes into tissuelyser or GeneGrinder.** Homogenize the samples for 3-10min at 30~50 Hz.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x *g*.

Difficult to grind sample Protocol:

1. Disrupt 50 mg plant or fungal tissue by bead-beat methods.
2. Add 0.6ml Buffer SOL to the sample and homogenize the samples for ~2 min at 30-50HZ.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x *g*.

SDS Lysis Protocol(High weight genomic DNA):

1. Disrupt plant or fungal tissue by Liquid nitrogen ground or other bead-beat methods.
2. Add 500µl Buffer SOL and 50µl Buffer SDS to a maximum of 50 mg (wet weight) or 15 mg (dried) disrupted plant or fungal tissue and vortex vigorously.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x *g*.

Manual Purify:

4. Transfer the 400µl of the supernatant into a new tube (not supplied) without disturbing the cell-debris pellet.
5. Add 30µl MagPure Particles and 600µl Buffer MPB to a new 1.5ml centrifuge tube. Pipette mix 20 times or shaking for 5 minutes. Place the tube on an Magnet Plate and allow beads to separate for 1 minutes. With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.
7. Add 600µl Buffer GW1, resuspend the beads by pipette mix 10 times or shaking for 1min. Place the tube to the magnetic stand for 1 minute, then remove the supernatant.
8. Add 600µl 80% ethanol , resuspend the beads by pipette mix 10 times or 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 pipette mix 10 times or shaking for 1min. Place the tube to the magnetic stand for 1 minute, then remove the supernatant.
10. Dry on air for 10 minutes.
11. Add ~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.
12. 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
Sample plate	600µl Buffer MPB 400µl Superntant from step 3.
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 96 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 BW1before 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 SOL and Buffer SDS.
- **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 (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 80% ethanol.
- **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.