

Magnetic Bead Method Plant DNA Extraction Kit

Project number: M669913

Storage conditions: room temperature.

Product Content:

Component	M669913
	96 preps
Buffer PLS	50 mL
Buffer ZB	55 mL
Buffer GW1 (concentrate)	80 mL
Buffer TB	10 mL
RNase A (10 mg/mL)	0.6 mL
Magbeads PN	2 x 1 mL

Product Introduction

This kit provides a simple and efficient solution for plant DNA extraction. After plant cells are physically broken, DNA from the lysate is bound to the surface of silica-coated magnetic beads in the presence of high salt. After rinsing, the DNA is eluted in Buffer TB or deionized water. The extraction rate is highly dependent on the sample type and the effect of cell breakage, and the extracted DNA can be used for downstream experiments such as second-generation sequencing and PCR testing.

Bring your own instruments and reagents

1. CWE2100 or CWE9600
2. 96 DW Plate, 8 tip Com, Spin tips pack
3. Anhydrous ethanol

Pre-experiment preparation and precautions

1. Before the first experiment add the specified amount of anhydrous ethanol to Buffer GW1 according to the instructions on the label of the reagent bottle;
2. Customers need to configure 75% ethanol for rinsing;
3. Magbeads PN should not be frozen or centrifuged at high speeds. Freezing and high speed centrifugation may cause irreversible damage to Magbeads.

procedure

manual operation

1. Crushing of plant material:

Program 1:

- 1) Weigh about 50–100 mg of fresh plant sample powder or 30 mg of dried sample powder

that has been well ground by liquid nitrogen, quickly transfer it into a centrifuge tube pre-filled with 400 μ L Buffer PLS and 5 μ L RNase A (10 mg/mL), quickly invert it to mix well and then centrifuge it instantly and place it in a thermostatic mixer at 70°C, 1600 rpm for 10 min. (Extraction of polysaccharide and polyphenol plants is enhanced by the addition of β -mercaptoethanol to Buffer PLS to a final concentration of 5%).

Program 2:

1) Add 50–100 mg of fresh plant material or 20 mg of dried plant material into a 2.0 mL centrifuge tube; 2) Grind the plant material with a glass until powdered after quick freezing with liquid nitrogen; add 400 μ L of Buffer PLS and 5 μ L of RNaseA (10 mg/mL) into the centrifuge tube, mix rapidly by inverting the mixture, and then put it into a thermostatic mixer at 70°C, 1600 rpm for 10 min. (For extracting polysaccharide polyphenol plants, add β mercaptoethanol into Buffer PLS to make its final concentration 5%, which can improve the effect of nucleic acid extraction. (For extraction of polysaccharides and polyphenols, add β -mercaptoethanol to the Buffer PLS to achieve a final concentration of 5% to improve nucleic acid extraction.)

Program 3:

1) Add 50 –100 mg of fresh plant material or 20 mg of dried plant material to a 2.0 mL centrifuge tube;
(2) Add 400 μ L of Buffer PLS, 5 μ L of RNaseA (10 mg/mL), and 1 steel ball to the centrifuge tube, and then immediately fix the tube in the Tissue Breaker and shake it to break;
3) After removing the centrifuge tube from the tissue breaker, centrifuge it instantaneously and place it in a thermostatic mixer at 70° C, 1600 rpm , for 10 min. 2. After centrifugation for 4 min at 12,000 rpm ($\sim 13,400 \times g$), transfer all of the supernatant to a new 1.5 mL centrifuge tube.
3. Add 550 μ L of BufferZB and 20 μ L of Magbeads PN to a 1.5 mL centrifuge tube, mix upside down for 30 s, centrifuge instantly, and allow to stand at room temperature for 5 min, mixing several times in between.
4. Centrifuge instantaneously, transfer the tube to a magnetic rack for 1 min (until the solution is clear) and discard the solution, avoiding contact with the beads during this time.
5. Remove the centrifuge tube from the magnetic rack, add 650 μ L of Buffer GW1, vortex for 10 s, resuspend the beads, and then fix the tube in a thermostatic mixer at 25° C and 1600 rpm for 2 min or vortex for 1 min.
6. Fix the centrifuge tube on a magnetic rack and let it stand for 1 min, after which the solution is fully discarded;
7. Repeat steps 5–6;
8. Remove the centrifuge tube from the magnetic rack, add 650 μ L of 75% ethanol, vortex for 10 s, resuspend the beads, and then place the tube on a thermostatic mixer at 25° C and 1600 rpm for 2 min or vortex for 1 min.
9. Fix the centrifuge tube on a magnetic rack and let it stand for 1 min, after which the solution is fully discarded.

10. Repeat steps 8-9;
11. Centrifuge the tube briefly and then remove the solution from the bottom of the tube again with a pipette and leave it at room temperature for 5-10 min to allow the ethanol to evaporate;
12. Add 100 μ L of BufferTB to the centrifuge tube, vortex the beads to fully suspend them in the eluate, and then place the beads on a thermostatic mixer at 65° C and 1600 rpm for 10 min, or incubate them for 10 min at 65° C in a water bath, vortexing four times during the incubation.
13. Fix the centrifuge tube on a magnetic rack and let it stand for 2 min. After the magnetic beads are fully adsorbed on the side wall of the centrifuge tube, transfer the eluate to a new centrifuge tube and store it at -20°C.

Matches CWE2100

1. Crushing of plant material:

Program 1:

1) Weigh about 50-100 mg of fresh plant sample powder or 30 mg of dried sample powder, which has been well ground by liquid nitrogen, and quickly transfer it into a centrifuge tube with 400 μ L Buffer PLS and 5 μ L RNase A (10 mg/mL), quickly invert and mix it well, then centrifuge it instantly and put it in a thermostatic mixer at 70°C, 1600 rpm for 10 min. (Extraction of polysaccharide and polyphenol plants is enhanced by the addition of β -mercaptoethanol to Buffer PLS at a final concentration of 5%).

Program 2:

1) Add 50 -100 mg of fresh plant material or 20 mg of dried plant material to a 2.0 mL centrifuge tube; 2) Quick-freeze with liquid nitrogen and then grind the plant material well with a glass until powdered;

3) Add 400 μ L of Buffer PLS and 5 μ L of RNaseA (10 mg/mL) to the centrifuge tube, mix quickly by inverting the tube, and place it in a thermostatic mixer at 70° C, 1600 rpm, for 10 min. (Adding β -mercaptoethanol to the Buffer PLS to achieve a final concentration of 5% in the extracted polysaccharide-polyphenol plants improves nucleic acid extraction).

Program 3:

- 1) Add 50-100 mg of fresh plant material or 20 mg of dried plant material to a 2.0 mL centrifuge tube;
 - 2) Add 400 μ L of Buffer PLS, 5 μ L of RNase A (10 mg/mL), and 1 steel ball to the centrifuge tube, then immediately fix the tube in the tissue breaker and shake it;
 - 3) After removing the tube from the Tissue Breaker, centrifuge it instantaneously and place it in a thermostatic mixer at 70° C, 1600 rpm , for 10 min. 2. After centrifugation at 12,000 rpm (~13,400 x g) for 4 min, transfer all the supernatant into a 96 DW deep well plate;
3. Add reagents to the 96 DW deep well plate according to the table below:

placement	Reagents and dosage
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1&7Colume	Lysate: ALL Buffer ZB: 550 μ L Magbeads PN: 20 μ L
2&8Colume	Buffer GW1: 650 μ L
3&9Colume	Buffer GW1: 650 μ L
4&10Colume	75% ethanol: 650 μ L
5&11 Colume	75% ethanol: 650 μ L
6&12Colume	Buffer TB: 100 μ L

4. Place the 96 DW deep well plate and the magnetic bar set in the CWE2100 instrument and run the Cowin Plant program.

5. After about 50 min, remove the deep-well plate and magnetic sleeve. Transfer the lysis products in columns 6 & 12 to a 1.5 mL centrifuge tube and store at -20°C . Match with CWE9600

1. Program 1:

1) Weigh about 50–100 mg of fresh plant sample powder or 30 mg of dried sample powder, which has been well ground by liquid nitrogen, and quickly transfer it into a centrifuge tube containing 400 μ L of Buffer PLS and 5 μ L of RNase A (10 mg/mL), quickly invert the mixture, then centrifuge it instantly, and then put it in a thermostatic mixer at 70°C , 1600 rpm for 10 min ((Addition of 5% β -mercaptoethanol to Buffer PLS for polysaccharide and polyphenol plants improves nucleic acid extraction).

Program 2:

1) Add 50–100 mg of fresh plant material or 20 mg of dried plant material to a 2.0 mL centrifuge tube;

2) Grind the plant material well to a powdered form using a glass after flash freezing with liquid nitrogen;

3) Add 400 μ L of Buffer PLS and 5 μ L of RNase A (10 mg/mL) to the centrifuge tube, mix quickly by inverting the tube, and then place it in a thermostatic mixer at 70°C , 1600 rpm, for 10 min. (Addition of 5% β -mercaptoethanol to Buffer PLS in the extraction of polysaccharide and polyphenol plants improves the extraction of nucleic acids).

Program 3:

1) Add 50–100 mg of fresh plant material or 20 mg of dried plant material to a 2.0 mL centrifuge tube;

2) Add 400 μ L of Buffer PLS, 5 μ L of RNase A (10 mg/mL), and 1 steel ball to the centrifuge tube, then immediately fix the tube in the tissue breaker and shake it;

3) Remove the tube from the tissue breaker and centrifuge it instantaneously in a thermostatic mixer at 70°C , 1600 rpm for 10 min.

2. After centrifugation at 12,000 rpm ($\sim 13,400 \times g$) for 4 min, transfer all supernatants to a 96 DW deep well plate;

3. Add reagents to the 96 DW deep well plate according to the table below:

placement	Reagents and dosage
Plate 1	Lysate: ALL Buffer ZB: 550 μ L Magbeads PN: 20 μ L
Plate 2	Buffer GW1: 650 μ L
Plate 3	Buffer GW1: 650 μ L
Plate 4	75% ethanol: 650 μ L
Plate 5	75% ethanol: 650 μ L
Plate 6	Buffer TB: 100 μ L

- Place the 96 DW deep well plate and the magnetic bar set in the CWE9600 instrument and run the program.
- After about 50 min, remove the plate and magnetic sleeve. Transfer the lysis products from Plate 6 to a 1.5 mL centrifuge tube and store at -20°C .