Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Magbead Plant DNA Kit

Product Number: EK0303

Shipping and Storage

Room temperature (15-30℃).

Component

Component	EK0303
Buffer PLS	60mL
Buffer ZB	55mL
Buffer GW1(concentrate)	80mL
Buffer TB	10mL
RNase A (10 mg/mL)	0.6mL
Magbeads PN	2×1mL

Description

This kit provides a simple and efficient method for extracting plant DNA. After plant cells are physically broken, the DNA in the lysate binds to the surface of silicon-based coated magnetic beads in the presence of high salt. After rinsing, the DNA is washed in Buffer TB or deionized water. The extraction yield is closely related to the sample type and cell fragmentation effect, and the extracted DNA can be used for downstream experiments such as second-generation sequencing and PCR testing.

Self provided instruments and reagents

1. DNA Extractor.
2. 96 DW Plate, 8 tip Comb, 96 Tip comb.
3. absolute ethanol.

Note

1. Add the specified amount of anhydrous ethanol to Buffer GW1 according to the instructions on the reagent bottle label before the first experiment.
2. The customer needs to configure 75% ethanol for rinsing.
3. Magbeads PN strictly prohibits freezing and high-speed centrifugation. Freezing and high-speed centrifugation may cause irreversible damage to Magbeads.

Protocol

1. Manual extraction process:

1.1. Sample pretreatment (liquid nitrogen grinding or homogenizer grinding and homogenization).
1.2. Add Buffer PLS and RNase A; Incubate at 70 ℃, centrifuge to obtain supernatant.
1.3. Add magnetic beads and buffer ZB, bind the magnetic beads, discard the supernatant.
1.4. Rinse Buffer GW1 twice and discard the solution.
1.5. Rinse twice with 75% ethanol and discard the solution.
1.6. Buffer TB elution, collect solution for future use.

2. Automated extraction process of nucleic acid extractor:

2.1. Sample pretreatment (liquid nitrogen grinding or homogenizer grinding and homogenization).
2.2. Add Buffer PLS and RNase A; Incubate at 70℃, centrifuge to obtain supernatant.
2.3. Invert and mix pre packaged reagents, centrifuge or shake gently to avoid liquid accumulation.

2.4. Transfer all the supernatant to a 96 DW deep well plate and add the corresponding reagents to the plate as required.
2.5. Insert the instrument and run the program.
2.6. Transfer nucleic acid to obtain purified DNA.

Fragmentation of plant materials

1. Option 1

1.1. Weigh about 50-100mg of fresh plant sample powder that has been thoroughly ground with liquid nitrogen or about 30 mg of dried sample powder, quickly transfer it to a centrifuge tube pre filled with 400-600μL Buffer PLS and 5μL RNase A (10 mg/mL), quickly invert and mix, centrifuge instantly, and place it in a constant temperature mixer at 70 ℃, 1600 rpm, for 10 minutes.

Note: Adding β - mercaptoethanol to Buffer PLS to extract polysaccharides and polyphenols from plants at a final concentration of 5% can improve the nucleic acid extraction efficiency.

2. Option 2:

2.1. Add 50-100mg of fresh plant material or 20 mg of dried plant material to a 2.0mL centrifuge tube;
2.2. After rapid freezing with liquid nitrogen, use a grinding pestle to thoroughly grind the plant materials into powder;
2.3. Add 400-600μL of Buffer PLS and 5μL of RNase A (10 mg/mL) to the centrifuge tube, quickly invert and mix, and place in a constant temperature mixer at 70℃, 1600 rpm, for 10 minutes.

Note: Adding β - mercaptoethanol to Buffer PLS to extract polysaccharides and polyphenols from plants at a final concentration of 5% can improve the nucleic acid extraction efficiency.

3. Option 3

3.1. Add 50-100 mg of fresh plant material or 20 mg of dried plant material to a 2.0mL centrifuge tube;
3.2. Add 400-600μL Buffer PLS, 5μL RNase A (10 mg/mL), and 1 steel ball to the centrifuge tube, and immediately fix the centrifuge tube in the tissue disruptor for shaking and crushing;
3.3. After removing the centrifuge tube from the tissue disruptor, centrifuge it instantly and place it in a constant temperature mixer at 70℃, 1600 rpm, for 10 minutes.

Note: Adding β - mercaptoethanol to Buffer PLS to extract polysaccharides and polyphenols from plants at a final concentration of 5% can improve the nucleic acid extraction efficiency.

Manual operation

1. After centrifugation at 12000 rpm (~13400 × g) for 4 minutes, transfer all the supernatant to a new 1.5mL centrifuge tube. Add 550μL Buffer ZB and 20μL Magbeads PN to a 1.5mL centrifuge tube, invert and mix for 30 seconds, and centrifuge instantly,
2. Let it stand at room temperature for 5 minutes, then invert and mix several times during this time. Instantaneous centrifugation, transfer the centrifuge tube to a magnetic rack for adsorption for 1 minute (until the solution becomes clear), then discard the solution and avoid contact with magnetic beads during this period.
3. Remove the centrifuge tube from the magnetic frame, add 650μL Buffer GW1, vortex and mix for 10 seconds, resuspend the magnetic beads, and then fix the centrifuge tube on a constant temperature mixer at 25℃ and 1600 rpm, shake and mix for 2 minutes or vortex and mix for 1 minute.
4. Fix the centrifuge tube on the magnetic frame and let it stand for 1 minute, then discard the solution thoroughly;
5. Repeat steps 3-4;
6. Remove the centrifuge tube from the magnetic frame, add 650μL of 75% ethanol, vortex and mix for 10 seconds, resuspend the magnetic beads, and then fix the centrifuge tube on a constant temperature mixer at 25 ℃ and 1600 rpm, shake and mix for 2 minutes or vortex and mix for 1 minute.
7. Fix the centrifuge tube on the magnetic frame and let it stand for 1 minute, then discard the solution thoroughly.
8. Repeat steps 6-7.
9. After briefly centrifuging the centrifuge tube, use a pipette to remove the bottom solution of the tube again, and then let it stand at room temperature for 5-10 minutes to allow ethanol to evaporate completely;

10. Add 100μL of Buffer TB to the centrifuge tube, suspend the magnetic beads fully in the eluent during vortex shaking, and then place them on a constant temperature mixer at 65℃ and 1600 rpm for 10 minutes of shaking and cracking, or incubate in a 65℃ water bath for 10 minutes, during which vortex shaking is performed 4 times.
11. Fix the centrifuge tube on the magnetic frame and let it stand for 2 minutes. After the magnetic beads are fully adsorbed on the side wall of the centrifuge tube, transfer the eluent to a new centrifuge tube and store it at -20℃ for later use.

Matching with DNA Extractor for automated extraction

1. After centrifugation at 12000 rpm (~13400 ×g) for 4 minutes, transfer all the supernatant to a 96 DW deep well plate.
2. Add reagents to the 96 DW deep hole plate according to the table below:

Position	Reagent and dosage
Plate 1	Lysate: ALL
	Buffer ZB: 550μL
	Magbeads PN: 20μL
Plate 2	Buffer GW1: 650μL
Plate 3	Buffer GW1: 650μL
Plate 4	75% ethanol: 650μL
Plate 5	75% ethanol: 650μL
Plate 6	Buffer TB: 100μL

3. Place the 96 DW deep hole plate and magnetic rod sleeve into the DNA Extractor instrument and run the program.
4. After about 50 minutes, the program runs and the deep hole plate and magnetic sleeve are removed. Transfer the pyrolysis products from columns 6&12 to a 1.5mL centrifuge tube and store at -20℃ for future use.

Result Analysis

The DNA concentration should be determined by measuring the absorbance at 260nm (A260) using a spectrophotometer. DNA has a significant absorption peak at 260nm, and the absorbance reading should be between 0.1 and 1.0. If it is not within this range, the sample needs to be diluted or concentrated. At 260nm, one unit of absorbance corresponds to 50μg of DNA per milliliter (A260=1 → 50μg/mL). The residual magnetic beads in the eluent may affect the A260 reading, but will not affect the performance of downstream DNA.

DNA sample concentration=50μg/mL × (A260-A320) × dilution factor

Total DNA amount=concentration x sample volume (mL)

The purity of DNA is determined by calculating the ratio of the corrected absorbance at 260nm to 280nm (subtracting the absorbance reading at 320nm to correct for the presence of magnetic particles), i.e. (A260-A320)/(A280-A320). The A260/A280 ratio of pure DNA is 1.7-1.9.