NanoMag Plant and Algae DNA Isolation Kit

Catalog Number: NA2012-01

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

NanoMag DNA Extraction Kit NA2012-01 is specially designed for purification of total DNA from Plants and Algae. The pretreatment method of plant samples will directly affect the isolation efficiency and the integrity of the DNA thus it is important to ensure that the sample pretreatment process is performed at low temperature and to sufficiently allow for cell disruption. Read procedure carefully before starting.

2. Test Principle

NanoMag DNA Extraction Kit comes with proprietary magnetic beads and a specially formulated buffer. The purified DNA can then be effectively eluted with TE or EL and is be ready for use in PCR or other enzymatic reactions or storage at -20°C. The procedures can be fully automated on the magnetic particle processor instrument and ease of use.

3. Applications

This kit is sufficient for 50 DNA isolations based on 200mg fresh plant, 1ml algae culture or 50mg dry seeds.

4. Components Provided in This Kit

Component Name	Volumes	Storage
Buffer TS	20mL	15–25°C
Buffer LWB*	30mL	15–25°C
Buffer WB	30mL	15–25°C
Mag Beads	1.5mL	2-8°C (upon arrival)
Buffer EL	5mL	15–25°C

* Buffer LWB contains chaotropic salts which are irritants. Please handle with appropriate laboratory safety measures and wear gloves.

* Storage period: 12 months

5. Storage and stability

Store Mag Beads at 4–8°C upon arrival, and the rest of the kit can be stored at room temperature (15–25°C). Freeze and violent centrifugation should be avoided. Buffer LWB must be kept away from light. Check Buffer TS and Buffer LWB for precipitate before use and re-dissolve at 37°C if necessary. Stability is guaranteed till expiry if properly stored and handled according to instructions.

6. User Supplied Materials

- Nuclease-free 1.5-2ml microcentrifuge tubes
- Vortex
- Incubator
- Centrifuge
- Liquid nitrogen, abrader or tissue homogenizer
- Magnetic separation device (Sold Separately)
- 75% ethanol v/v with 25% sterile water
- Elution buffer: EL, TE or 10mM Tris-HCl (pH=8.0)

7. Sample Preparation

1. Sample Pretreatment: The grinding method of plant samples will affect the yield of DNA and the integrity of the fragments. It is recommended to use liquid nitrogen grinding or tissue homogenization method to obtain high purity products.

- For plants:
  - The plant leaves are ground with liquid nitrogen or homogenized using a homogenizer.
  - Transfer 50-100mg of the powdered sample to a clean microcentrifuge tube and add 400µl of Buffer TS.
  - Incubate at 70°C for 10 minutes.
  - Centrifuge the sample at 10,000xg for 5 minutes.

- For Algae:
  - Algae culture is added to a 1.5-2mL tube and pelleted by centrifugation and remove media.
  - Add 400µl of Buffer TS to pellet.
  - Fully resuspend the algae pellet by vortexing for 1 minute.
  - b. Sonicate using a water bath sonicator at maximal power for 10 minutes.
  - Incubate at 70°C for 10 minutes.

- Centrifuge the sample at 10,000xg for 5 minutes.

2. Carefully transfer 300μl supernatant to a new microcentrifuge tube, add 600μl Buffer LWB into the microcentrifuge tube.

3. Fully resuspend Mag Beads by vortexing for 1 minute. Add 30μl Mag Beads, and completely resuspend the magnetic particles by vortexing or pipetting up and down for 10 times. Incubate at room temperature for 10 minutes. Mix the tube every 2–3 minutes during incubation to help lysis and binding.

Note: Mag Beads tend to settle to the bottom, it is very important to resuspend the beads thoroughly before use to ensure a homogeneous mix of this reagent is transferred into each well to avoid the difference between wells.

4. Place the tube onto a magnetic stand for 1 minute and aspirate the supernatant with pipette carefully without aspirating the magnetic beads.

Note: Aspiration should be performed while the plate is situated on the magnetic stand. Be careful not to disturb or aspirate the beads pellet. It is important to completely remove all of the supernatant.

5. Remove the tube from the magnetic stand and add 600μl Buffer WB to the tube. Completely resuspend the magnetic beads by vortexing.

6. Place the plate onto a magnetic stand for 1 minute or until the solution clears. Aspirate the cleared supernatant with a pipette carefully without aspirating the magnetic beads and then discard the supernatant.

7. Add 600μl 75% ethanol to the tube. Completely resuspend the magnetic beads by vortexing.

8. Place the plate onto a magnetic stand for 1 minute or until the solution clears. Aspirate the cleared supernatant with a pipette carefully without aspirating the magnetic beads and then discard the supernatant.

9. Wash the magnetic beads again by repeating the step 7 and 8.

10. Remove any trace of liquid with pipette tips. Air-dry the magnetic beads by placing the tube at room temperature for 5-10 minutes.

Note: Do not vacuum dry, and excessive drying can lower the recovery rate.

11. Add at least 50µl Elution Buffer (TE or EL) and resuspend the magnetic beads by pipetting up and down for 10 times or vortexing. Incubate at 65°C for 10 minutes.

Note: Elution efficiency is depending on pH. The maximum elution efficiency is achieved between pH7.0 and 8.5. When using water for elution, make sure the pH is within this range, and store DNA at -20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted with buffer TE (10mM Tris-HCl, 1mM EDTA, pH8.0) but the EDTA may inhibit subsequent enzymatic reactions.

12. Place the plate onto a magnetic stand to magnetize the beads. The solution should be cleared after all magnetic beads are completely pelleted.

13. Transfer the cleared supernatant into a new tube.

8. Results

11.1 Calculating the concentration of DNA

- Analyze the concentration of the DNA using absorbance measurement with a spectrophotometer using 260nm wavelength. A_{260} of 1.0 = 50µg/ml pure dsDNA. So if the OK reading is 0.3 at OD 260 the concentration = 0.3 x 50µg/ml or 15ug/ml or 15ng/ul.

9. General Instructions

Problem 1. Little or no nucleic acid in the eluate

Cause	Solution
Sample grinding is not sufficient	The sample was thoroughly ground with liquid nitrogen or the sample was homogenized with a homogenizer.
Sample amount is too much	Reducing the amount of sample (especially polysaccharides) or increase the amount of Buffer TS.
Inadequate sample lysis	After adding Buffer TS, completely resuspend the sample by vortexing, and avoid the sample gathering together.
***Buffer TS* and *Buffer LWB* crystallization**	re-dissolve at 37°C before use.
75% ethanol prepared incorrectly	Check if 75% ethanol were diluted with the correct volume from 96-100% ethanol. Repeat the purification procedure with new samples.
75% ethanol prepared with low-percentage ethanol	Do not use denatured alcohol, which may contain other substances such as methanol or methyl ethyl ketone.
Inappropriate elution buffer	DNA will only be eluted in the presence of low salt buffer (e.g.10 mM Tris-HCl, 1mM EDTA, pH 8.0) or water. Check the pH and salt concentration of the elution buffer.
Over dried Mag Beads	Mag Beads must be fully resuspended before use or in the purification procedure.

Problem 2. DNA does not perform well in downstream enzymatic reactions

Cause	Solution
Little or no DNA in the eluate	See "Little or no nucleic acid in the eluate" for possible reasons. Increase the amount of eluate added to the reaction if possible.
Some degradation of genomic DNA	Repeat the purification procedure with well-preserved or fresh samples.
concentration of DNA in the samples	Increase sample volume and the volume of buffer, then repeat the purification procedure.
Not completely remove Buffer LWB	***Buffer LWB*** contains chaotropic salts which may inhibit subsequent enzymatic reactions, wash Mag ***Beads*** 2 times with ***Buffer WB***.

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Our mission is to:

- Enhance detection technologies
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- Enable impactful research discoveries

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