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 Elution Buffer and is ready for use in PCR, other 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 100 DNA isolations based on 200mg fresh plant, 1ml algae culture or 50mg dry seeds.

4. Components Provided in This Kit

Component Name	Volumes	Storage
Suspension Buffer	2 x 20mL	15–25°C
Lysis Solution**	30mL	15–25°C
Mag Beads	2 mL	2-8°C (upon arrival)
Wash Buffer 1**	38mL	15–25°C
Wash Buffer 2**	20mL	15–25°C
Proteinase K	2 x 1mL	15–25°C
Elution Buffer	6mL	15–25°C

* Lysis Buffer and Wash Buffer 2 contains chaotropic salts which are irritants. Please handle with appropriate laboratory safety measures and wear gloves.

** Before using for the first time, add the appropriate volume of ethanol (96%-100%) as indicated on the bottle to the Lysis Solution, Washing Buffer I, and Washing buffer II. Mark the bottles, shake and store at room temperature.

5. Storage and stability

Store Mag Beads and proteinase K at 4–8°C upon arrival, and the rest of the kit can be stored at room temperature (15–25°C). Freezing and violent centrifugation should be avoided. Check Lysis 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)
- 96-100% ethanol

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 Suspension Buffer.
  - Add 20µl of Proteinase K
  - Incubate at 37°C for 30 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 Suspension Buffer to pellet.
    - Fully resuspend the algae pellet by vortexing for 1 minute.
    - Sonicate using a water bath sonicator at maximal power for 10 minutes.
    - Add 20µl of Proteinase K
    - Incubate at 37°C for 30 minutes.
    - Centrifuge the sample at 10,000xg for 5 minutes.

2. Carefully transfer 200µl supernatant to a new microcentrifuge tube and 700µl Lysis Buffer into the microcentrifuge tube.

3. Fully resuspend Mag Beads by vortexing for 1 minute. Add 20µl 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 tube 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 700µl Wash Buffer 1 to the tube. Completely resuspend the magnetic beads by vortexing.

6. Place the tube 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 700µl Wash Buffer 2 to the tube. Completely resuspend the magnetic beads by vortexing.
8. Place the tube 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. 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.

10. Add at least 50µl Elution Buffer and resuspend the magnetic beads by pipetting up and down 10 times or by vortexing. Incubate at room temperature for 10 minutes.

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

12. Transfer the cleared supernatant into a new tube.

Note: Store purified DNA at -20°C for short term and -70°C for long term storage.

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. Example: If the reading is 0.3 at OD 260 the concentration = 0.3 x 50µg/ml = 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 Suspension Buffer.
Inadequate sample lysis	After adding Suspension Buffer, completely resuspend the sample by vortexing, and avoid the sample from settling.
Lysis Buffer and Wash Buffer II 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.
Did not completely remove Wash Buffer	Wash Buffer II contains chaotropic salts which may inhibit subsequent enzymatic reactions, wash Mag Beads 2 times with Wash Buffer I.

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

Contact Us
3913 Todd Lane, Suite 310
Phone: 512- 333-1330
Email: sales@attogene.com
Web: www.attogene.com
NA2012-01.V5_20210715