Plant and Algae DNA Isolation Kit

Catalog Number: NA2012

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

1. Background

NA2012 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

NA2012 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	120mL	15–25°C
Lysis Solution**	50mL	15–25°C
Mag Beads	20mg/mL	15–25°C
Wash Buffer 1**	50mL x 2	15–25°C
Wash Buffer 2	100mL	15–25°C
Proteinase K	25mg	15–25°C
RNase A	250ul	-20°C
Poly A (Carrier RNA)	2mg	15–25°C
Elution Buffer	20mL	15–25°C

* Lysis Buffer and Wash Buffer 1 contains chaotropic salts which are irritants. Please handle with appropriate laboratory safety measures and wear gloves.

5. Before You Begin

1. Completely dissolve Proteinase K into 1,250ul of Elution Buffer. For short term storage, dissolved Proteinase K can be stored at 4°C. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at -20°C.

2. Dissolve Poly(A) with 500ul of Elution Buffer, gently mix with vortex mixer.

3. Mix dissolved Poly(A) solution into Lysis Solution. NOTE: If poly(A) may interfere in your downstream applications this step can be skipped.

4. Add 50mL of absolute ethanol as indicated on the bottle to the wash buffer 1. Mark the bottle, shake and store at room temperature.

6. Storage and stability

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.

7. User Supplied Materials

- Nuclease-free 1.5-2ml microcentrifuge tubes
- Vortex
- Incubator
- Water Both Sonicator
- Centrifuge
- Liquid nitrogen, abrader or tissue homogenizer
- Magnetic separation device (Sold Separately)
- 96-100% ethanol

8. Sample Preparation

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 500μl of Suspension Buffer.
- Add 10μl of Proteinase K.
- Incubate at 55°C for 30 minutes.

For Algae:

- Suspended algae is added to a 1.5-2mL tube and pelleted by centrifugation and remove liquid.

  NOTE: If algae is floating, remove liquid from below the surface of the algae.
- Add 500μl of Suspension Buffer to the algae.
- Fully resuspend the algae by vortexing for 1 minute.
- Sonicate using a water bath sonicator at maximal power for 10 minutes.
- Add 10μl of Proteinase K.
- Incubate at 55°C for 30 minutes.

1. Carefully transfer 200μl of the Proteinase K treated plant or algae solution from above into a new microcentrifuge tube and add 200μl Lysis Buffer (containing poly A) into the microcentrifuge tube and vortex for 10 seconds.
2. Add 400ul of absolute alcohol and mix thoroughly by vortexing.
3. Add 200μl of vortexed Mag Beads and mix for 30 seconds.

4. **Note**: Mag Beads tend to settle to the bottom, it is 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
5. Place the tube onto a magnetic stand for 1 minute and aspirate the supernatant with pipette carefully without aspirating the magnetic beads.
6. **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.
7. Remove the tube from the magnetic stand and add 700µl Wash Buffer 1 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. (Repeat steps 5 and 6).
10. Remove the tube from the magnetic stand and add 700µl Wash Buffer 2 to the tube. Completely resuspend the magnetic beads by vortexing.
11. 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.
12. (Repeat steps 8 and 9).
13. Remove the tube from the magnetic stand and add 300µl Suspension Buffer to the tube. Gently resuspend the magnetic beads by vortexing.
14. Add 2.5µl of RNase A and mix.
15. Incubate for 15 minutes at room temperature.
16. 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.

17. Remove the tube from the magnetic stand and add 300µl Suspension Solution to the tube. Completely resuspend the magnetic beads.
18. 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.
19. (Repeat steps 15, 16 and 17)
20. Completely remove any trace of liquid with pipette tips.
21. Add 50-100µl Elution Buffer and resuspend the magnetic beads by pipetting up and down 10 times or by vortexing. Incubate at room temperature for 10 minutes.
22. Place the tube onto a magnetic stand to magnetize the beads. The solution should be cleared after all magnetic beads are completely pelleted.
23. 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.

9. Results

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.

10. General Instructions

Problem 1. Little or no nucleic acid in the eluate

Cause	Solution
Sample homogenization is not sufficient	The sample was thoroughly homogenized.
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 crystallization	re-dissolve at 37°C before use.
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 1 contains chaotropic salts which may inhibit subsequent enzymatic reactions, wash Mag Beads 2 times with Wash Buffer 1.

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

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