AttoTector NanoMag Plant RNA Kit

Catalog Number: NA2014-01

(50 reactions based on 100 mg of plant tissue)

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

1. Introduction

The NanoMag Plant RNA Kit is optimized for purification of total RNA from a wide variety of plant samples which rich in polysaccharides and phenol. It comes with proprietary magnetic beads and a specially optimized buffer. Toxic solvents such as phenol, chloroform are not needed in the process. The eluted RNA is high-quality and is ready for all downstream applications, such as RT-PCR, Northern blotting, differential display. The procedures can be fully automated on the magnetic particle processor instrument for ease of use.

4. Kit Contents

- Buffer RLT ..... 30 mL
- Buffer BC ..... 20 mL
- Buffer PBR2 ..... 35 mL
- Buffer PBR3 ..... 8 mL
- DNase I ..... 1500 U/mL
- DNase Buffer ..... 0.5 g/mL
- Wash Buffer ..... 25 mL
- Buffer RW2 ..... 30 mL
- NanoMag ..... 1 mL x 2
- RNase-free Water ..... 10 mL

5. Storage and Stability

Store NanoMag Beads and lyophilized DNase I at 4-8°C upon arrival, the rest of the kit can be stored at room temperature (15-25°C).

Stability is guaranteed till expiry if properly stored and handled according to instructions.

6. Equipment and Reagents Needed (not provided)

6.1 Equipment

- Nuclease-free 2 mL microcentrifuge tubes
- Vortex
- Mortar and pestle or Rapid bead-beater
- Microcentrifuge
- Magnetic separation device (Sold Separately)

6.2 Reagents

- Absolute Ethanol (96%-100% v/v)
- Liquid Nitrogen

7. Reagent Preparation

- Add 400 μl DNase I Buffer to the tube of lyophilized DNase I
- Add 15 mL ethanol (96–100%v/v) to ***Buffer PBR2***

8. Sample Preparation

- Add 600μl ***Buffer RLT*** into a RNase-free 2ml microcentrifuge tube. Immediately place the tissue in liquid nitrogen and grind thoroughly with a mortar and pestle or other homogenate methods. Add a maximum of 100mg tissue powder to the tube with ***Buffer RLT***. Vortex vigorously.
- Centrifuge at maximum speed for 10minutes at room temperature. Carefully transfer 400μl supernatant to a new 2mL microcentrifuge tube.
- Add 1 volume of ***Buffer BC*** to the cleared lysate.
- Fully resuspend **NanoMag** ***Beads*** by vortexing for 1minute. Add 40μl **NanoMag** ***Beads***, and completely resuspend the magnetic particles by vortexing or pipetting up and down for 10 times. Incubate at room temperature for 5 minutes. Mix the tube every 2-3 minutes during incubation to help lysis and binding.

  Note: **NanoMag** ***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 tube to avoid the difference between tubes.
- 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.

- Remove the tube from the magnetic stand and add 700μl Buffer PBR2 into the tube. Completely resuspend the magnetic beads by vortexing for 1-2 minutes.
- 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.
- Remove the tube from the magnetic stand and add 144 μl Buffer PBR3 and 6 μl DNase I into the tube. Completely resuspend the magnetic beads by vortexing 2 minutes. Incubate at room temperature for 20 minutes to digest DNA.

  Note: Make sure the magnetic beads are totally immersed in the reaction mixture. DO NOT remove the supernatant before step
- Add 600 μl ***Buffer RW2*** into the tube. Completely resuspend the magnetic beads by vortexing for 1-2 minutes.
- 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.
- Remove the tube from the magnetic stand and add 500 μl Wash Buffer into the tube. Completely resuspend the magnetic beads by vortexing for 1-2 minutes.
- 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.
- Remove the tube from the magnetic stand and add 800 μl Absolute Ethanol (96—100%) into the tube. Completely resuspend the magnetic beads by vortexing 1-2 minutes.
- 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.
- Remove any trace of liquid with pipette tips. Air-dry the magnetic beads by placing the tube at room temperature for 5 minutes.

  Note: Do not vacuum dry, and excessive drying can lower the recovery rate.
- Add at least 50 μl RNase-Free ddH2O and resuspend the magnetic beads by pipetting up and down for 10 times or vortexing. Incubate at room temperature for 5 minutes.

  Note: Elution efficiency is depending on pH. The maximum elution efficiency is achieved between pH7.0 and 8.5. Make sure the pH of the RNase-Free ddH2O is within this range, and store RNA at -80°C as RNA may degrade in the absence of a buffering agent.
- Take 1.0±0.05g egg sample into a 10ml polystyrene centrifuge tube, add 3ml sample extraction solution (solution 5), shake it with shaker for 1min to dissolve it;
- Place the tube onto a magnetic stand to magnetize the beads. The solution should be cleared after all magnetic beads are completely pelleted. Transfer the cleared supernatant into a new

tube.

9. Troubleshooting Notes

Problem 1. Little or no nucleic acid in the eluate

Cause	Solution
Inappropriate handling of starting material	Extract with fresh plant materials.
Inefficient disruption or homogenization	Increase disruption or homogenization time.
Too much starting material	Reduce the amount of starting material.
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.
Overly-dried NanoMag Beads	If Beads are too extensively dried, wash the magnetic Particles again with 800μl absolute ethanol.
RNase contamination	Be certain not to introduce any RNases during the procedure or later handling.

Problem 2. DNA contamination in downstream experiment

Cause	Solution
Beads partially immersed in DNase reaction Mixture	Make sure the magnetic beads are totally immersed in the reaction mixture.
Too much starting material	In subsequent preparations, reduce the amount of starting material.

Problem 3. RNA does not perform well in downstream experiments

Cause	Solution
Little or no nucleic acid 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.
Alcohol present in the eluate	Elute RNA after the magnetic beads are thoroughly dried.
Salt present in the eluate	Remove wash buffer completely in each step.

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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