AttoTector

NanoMag Soil DNA Isolation Kit

Catalog Number NA2013-XX

ATTOGENE CORPORATION

Solutions for Life Science

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Introduction

NanoMag Soil DNA Extraction Kit(s) NA2013-XX is an excellent kit for isolating genomic DNA from a variety of environmental samples. NanoMag Soil DNA Extraction Kit(s) NA2013-XX utilizes our novel inhibitor removal reagents to efficiently remove humic acids and other inhibitors. This kit is intended for use with difficult soil types such as soils from forest, bedding, flowerpot, farmland and sludge. The isolated DNA has a high level of integrity and purity allowing for various downstream applications. The procedures can be fully automated on the magnetic particle processor instrument and ease of use.

Kit Contents:

Component Name	50 Prep Amount	100 Prep Amount	200 Prep Amount
Buffer LHA	40mL	80mL	160mL
Buffer PRE	5mL	10mL	15mL
Buffer LWB*	60mL	120mL	240mL
Buffer WB	40mL	80mL	160mL
Buffer SWP[1]	10mL	20mL	40mL
Glass Beads	25g	50g	100g
NanoMag Beads	1.5 mL	1.5 mL x2	1.5 mL x 4
Buffer EL	5 mL	10 mL	20 mL

#Number of reactions demonstrated is based on 250mg soil.

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

[1]Ethanol must be added prior to use into bottle labeled Buffer SPW

Storage and Stability

Store NanoMag 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 LHA and Buffer LWB for precipitate before use and re-dissolve at 65°C (Stability is guaranteed till expiry if properly stored and handled according to instructions).

User Supplied Materials

- Nuclease-free 1.5-2mL microcentrifuge tubes
- Vortex
- Magnetic separation device (Sold Separately)
- Absolute Ethanol (96%–100% v/v)

Purification Protocol:

1. Add 0.5g glass bead provided, 0.25g soil sample, and 800μl of ***Buffer LHA*** to a 2ml centrifuge tube, vortex 15min to mix thoroughly with a vortex.

Note: This kit is applicable for a common 0.25g of soil sample. For those soils that can absorb plenty of water, such as peat soil, clay and compost, please reduce soil samples accordingly. If using a bead beater to homogenize and lyse soil, please adjust the homogenization speed and time to obtain maximum DNA yields and integrity.

2. Centrifuge at 12,000rpm for 2 min at room temperature.

3. Carefully transfer the supernatant to a new 1.5 ml or 2 ml microcentrifuge tube. Add 100μl ***Buffer PRE***, vortex to mix.

4. Centrifuge at 12,000rpm for 3 min at room temperature. Carefully transfer the supernatant to a new 1.5ml or 2ml microcentrifuge tube.

5. Fully resuspend ***NanoMag Beads*** by vortexing for 1 minute. Add 2 volumes of ***Buffer LWB*** and 30μl ***NanoMag 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: ***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 well to avoid the difference between wells.

6. 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.

7. Remove the tube from the magnetic stand and add 800μl ***Buffer WB*** into the tube. Completely resuspend the ***NanoMag Beads*** by vortexing for 1-2min.

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 the tube from the magnetic stand and add 600µl ***Buffer SPW*** to the tube. Completely resuspend the ***NanoMag Beads*** by vortexing.

10. 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.

11. Wash the magnetic beads again by repeating step 9 and 10.

12. Remove any trace of liquid with pipette tips. Air-dry the magnetic beads by placing the tube at room temperature for 10-20 minutes.

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

13. Add **at least** 30µl **Elution Buffer** (EL or TE) 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 pH 7.0 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 TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH8.0) but the EDTA may inhibit subsequent enzymatic reactions.

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

15. Transfer the cleared supernatant into a new tube.

Results

Calculating the concentration of DNA

Analyze the concentration of the DNA using absorbance measurement with a spectrophotometer using 260nm wavelength. A260 of 1.0 = 50µg/ml pure dsDNA. For example, if the reading is 0.3 at OD 260 the concentration = 0.3 x 50µg/ml or 15ug/ml or 15ng/ul.

Troubleshooting Notes

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	NanoMag Beads must be fully resuspended before use or in the purification procedure.

Troubleshooting Notes (cont.)

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

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 **NanoMag *Beads*** 2 times with ***Buffer WB***.

Who we are

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness of plants, animals and the environment 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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