NucleoGene Environmental DNA Extraction Kit

Instructions for Use

Release Date– 01.12.2023

REF

NGE042– 50 Test
100 Test
250 Test

For the isolation of Environmental samples.
For nucleic acid isolation and purification.
For research purposes only.
Not suitable for diagnostic use.

Kit Contents

	Material Supplied	50 Test	100 Test	250 Test
1.	Lysis Buffer	45 ml	90 ml	225 ml
2.	Binding Buffer	55 ml	110 ml	140x2 ml
3.	Precipitation Buffer	4 ml	8 ml	20 ml
4.	Wash Buffer I*	15 ml	30 ml	75 ml
5.	Wash Buffer II*	15 ml	30 ml	38x2 ml
6.	Elution Buffer	10 ml	20 ml	75 ml
7.	Spin Columns	50	100	250
8.	Collection Tubes	50	100	250
9.	Bashing Bead Tubes	50	100	250
10.	User Manual	1	1	1

* For the preparation of the solutions, follow the instructions on the bottles before use.

† Deposition may occur during storage. Heat to solve the precipitate before use.

Storage & Durability

All components of the kit should be stored at room temperature (15-25 ° C). Do not expose the kit to direct sunlight. The kit can be stored for 12 months without any loss of performance when used under these conditions.

Purpose of Use

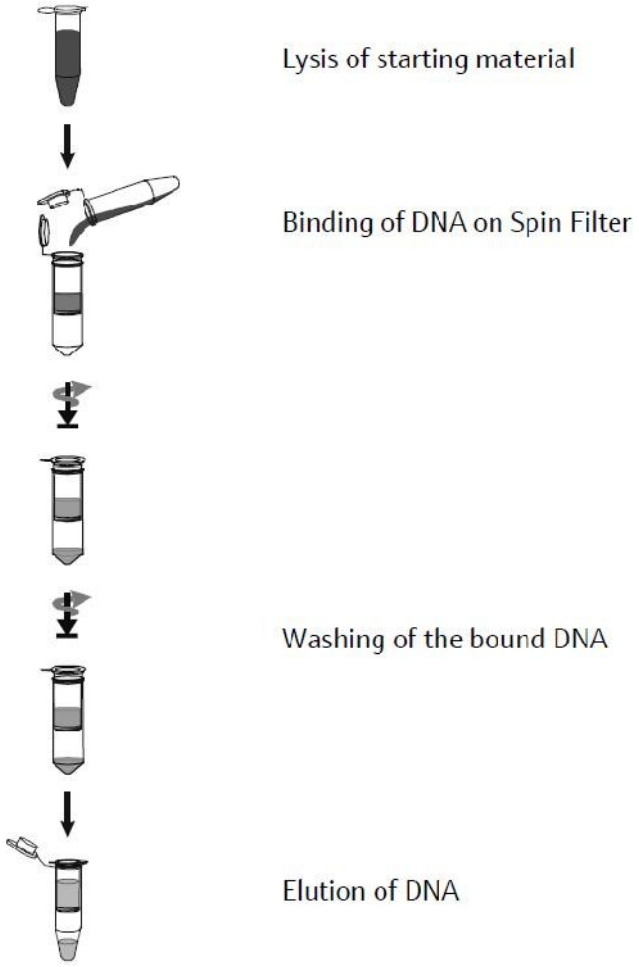
NucleoGene Environmental DNA Extraction Kit provides a convenient and rapid method for the detection of microorganisms from soil samples. All types of soil samples can be processed with this kit, including common soil samples and difficult soil samples with high humic acid content such as compost and manure. The kit removes all traces of humic acid and PCR inhibitors using the provided the Precipitation Solution. A simple and rapid spin column procedure is then used to further purify the DNA. Total genomic DNA can be isolated and purified from all the various microorganisms found in soil, such as bacteria, fungi and algae. The purified DNA is of the highest quality and is fully compatible with downstream PCR applications, as all humic acid substances and PCR inhibitors are removed during the isolation.

Product Usage Limits

- The NucleoGene Environmental DNA Extraction Kit intended for purification of DNA from saliva samples. It cannot be used for clinical purposes. It is up to the user's discretion whether the user is suitable for the specific experiment design.
- The ability of this kit to specifically isolate DNA from Environmental samples has been confirmed.
- Picking, transporting and storing the samples to be used is at least as sensitive as the purification and can be sensitive to the result.
- Designed for professional use only by trained personnel.
- Kit components with different lots should not be combined used together.

Introduction

NucleoGene Environmental DNA Extraction Kit provides a fast, simple and cost-effective method for the isolation of DNA from soil starting materials. The unique buffer system efficiently lysis cells and allows the nucleic acid to easily bind to the spin column filter. Pollutants such as salts, metabolites, soluble macromolecular and cellular components are removed in the wash step. Phenol extraction and ethanol precipitation are not required and high quality Nucleic Acid is eluted with Elution Buffer. NucleoGene Environmental DNA Extraction Kit suitable for a variety of routine applications including isolated DNA, Real-Time PCR, PCR, DNA sequence analysis, Digital PCR, NGS, Microarray and other enzymatic reactions.



Purified DNA with complete isolation should be used immediately or a freezer at -20 ° C should be preferred for long-term storage.

Warnings and Precautions

- All clinical specimens and residues and residues from them should be treated as potentially infectious and disposed accordingly.
- All samples should be prepared in Biosafety Level 1 or 2 areas or in Class II type Biosafety Cabins.
- Before and after work all surfaces should be clean a disposable paper towel daily with freshly prepared bleach.
- Do not forget to use laboratory safety devices such as disposable gloves, goggles, visors, disposable cuffs, disposable masks.
- If any of the kit components come into contact with your skin, wash them with plenty of water in no time. In case of contact with your mucus membrane, such as your eyes or mouth, wash the affected area with plenty of water, but do not forget to consult a physician.
- If possible, choose the pipette tips with filter.
- Some solutions in the kit contain guanidine salts. These salts form reactive compounds and toxic gases when mixed with bleach. Do

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not mix these solutions or the garbage that occurs during the isolation with the bleach.
- Keep the kit away from sources of contamination such as DNA and RNA, especially amplified nucleic acid.
- Do not mix the solutions with different lot numbers, or use the products of other companies instead.
- For more information, please refer to the Material Safety Data Sheet (MSDS) which you can request from www.nucleogene.com.

Other Materials Required

- Molecular Biology Degree(DNAse & RNAse free) ddH2O
- Bead Beater Device
- Ethanol (96-100%) Molecular Biology Degree
- RNase A (10 mg/ml); optional
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Microcentrifuge tubes (2.0 ml)
- Micropipette set and micropipette tips with sterile filter
- NucleoGene Homogenization Bead Tube

Notes Before You Begin

If the kit is stored in a non-conditioned environment, Solution Lysis Buffer may form precipitates at in cold weather. In this case, dissolve the solution Lysis Buffer bottle in the 50 ° C water bath until the precipitates are completely dissolved to dissolve the precipitate. Ensure that all solutions are at room temperature prior to the protocol and when the protocol is being applied, if not wait for room temperature.

Preparation of Solutions;

Wash Buffer I:
Add 10 mL of 96 % or absolute ethanol for 50 tests
Add 20 mL of 96 % or absolute ethanol for 100 tests
Add 50 mL of 96 % or absolute ethanol for 250 tests

Wash Buffer II:
Add 60 mL of 96 % or absolute ethanol for 50 tests
Add 120 mL of 96 % or absolute ethanol for 100 tests
Add 150 mL of 96 % or absolute ethanol per bottle for 250 tests

Protocol

Before starting DNA isolation, check that there are no leaks in the reagents. Gently shake the reagents to mix the solutions. If the reagents contain precipitates (especially Lysis solution), dissolve by heating at 50 °C.

Soil DNA Extraction

1. Add 0.25 grams of soil sample into a Homogenization Bead Tube (not-provided).

2. Add 850 ul of Lysis Buffer to the tube. Caution: If a sample has high water content, transfer the beads from bead tube to a 1.5 ml microcentrifuge tube (not provided), add wet sample to the empty bead tube, centrifuge at 12,000 x g for 5 minutes, and remove the liquid with a pipette tip. Transfer the glass beads back to the bead tube, and then add Lysis Buffer.

3. Secure the tube horizontally on a flat-bed vortex pad with tape or on a vortex microtube holder. Vortex at maximal speed for 10 minutes. Alternatively, beat for 40 seconds at 6M/S using commercial bead beaters according to recommendations from the manufacturer. Centrifuge the tube at 12,000 x g for 5 minutes.

4. Transfer up to 550 ul of supernatant to a 1.5 ml microcentrifuge tube (not provided). If removal of RNA is required, add 10 ul of DNase- free RNase A (10 mg/ml, not provided) to the tube, mix by pipetting 3 times, and incubate for 10 minutes room temperature.

5. Add 55 ml of Precipitation Buffer to the supernatant and mix by inverting the tube 5 times. Incubate the tube on ice for 5 minutes. Centrifuge the tube at 20,000 x g for 5 minutes.

6. Transfer 500 ml of supernatant, avoiding transfer of any of the pelleted material, to a clean 1.5 ml microcentrifuge tube (not provided). Caution: If the supernatant is unclear, transfer all solution to a new 1.5 ml microcentrifuge tube, centrifuge at 20,000 x g for 3 minutes to clarify the solution, and then transfer 500 ml of supernatant to a new 1.5 ml microcentrifuge tube.

7. Add 1000 ml of Binding Buffer to the supernatant and mix by pipetting 3 times. Load 600 ml solution into a spin column and centrifuge at 10,000 x g for 1 minute. Discard the flow through and add an additional 600 ml of solution to the spin column and centrifuge at 10,000 x g for 1 minute. Load the remaining solution to the column and centrifuge at 10,000 x g for 1 minute. Discard the flow through.

   Optional: if the sample has high content of humic acids, add 550 µl of Binding Buffer, wait for 2 minutes and centrifuge at 14,000 × g for 1 minute. Discard the flow through. Repeat this step once.

8. Apply 500 µL Wash Buffer I to the column and centrifuge at 8000 x g for 1 minute. Discard any remaining liquid and reinsert the spin column into the collection tube.

   Note: Inspect the column to ensure that all of the wash solution passes into the collection tube. If not all Wash Biffer I has passed, centrifuge for another minute.

9. Apply 500 µL Wash Buffer II to the column and centrifuge at 8000 x g for 1 minute. Discard any remaining liquid and reinsert the spin column into the collection tube

10. Centrifuge for 2 minutes at maximum speed to ensure that no ethanol residue remains in the spin column. Discard the collection tube.

11. Place the column in a 1.5 ml microcentrifuge tube (not provided).

12. Add 100 µl of pre-warmed at 70° C Elution Buffer to the center of white membrane. Optional: Add 35-50 µl of Elution Buffer instead if a higher concentration of DNA is desired. Wait for 2 minutes and then centrifuge at 10,000 × g for 1 minute.

13. Discard the spin column. The DNA is now ready for downstream applications. Purified DNA sample can be stored at 4° C for several days. For long-term storage, it is recommended to place samples at −20° C.

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Troubleshooting

Problem / probable cause	Comments and suggestions
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pelletun-lysed material. Reduce amount of starting material.
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Over-loading reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5 minutes or repeat elution step once again. Take a higher volume of Elution Buffer.
Insufficient mixing with Binding Solution	Mix sample with Binding Solution by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Too much Elution Buffer was used in the elution step	Elute the DNA with lower volume of Elution Buffer
Incorrect storage of starting material	Ensure that the starting material is frozen immediately after taking in liquid nitrogen or at -18 °C to -22° C! For long time storage continuously store at -78 °C to -82° C! Avoid thawing of the material.
Old starting material	Old material often contains degraded DNA. Repeat with fresh material.
Extracted DNA is contaminated with RNA	Perform an RNase A digestion.

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