NucleoGene Stool DNA Quick Extraction Kit

Instructions For Use

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REF

For isolation and purification of Deoxyribonucleic acid from fecal samples.
For research implementation only.
Not suitable for diagnostic use.
For professional use only.

NGE032– 50 Tests
100 Test
250 Test

Kit Contents

	Supplied Material	50 Tests	100 Tests	250 Tests
1.	Proteinase K*	1 vial	2 vial	1 vial
2.	Proteinase K Buffer	1 ml	2 ml	5 ml
3.	Lysis Buffer †	10 ml	20 ml	50 ml
4.	Inhibitor Removal Buffer †	50 ml	100 ml	250 ml
5.	Wash I Buffer*	15 ml	30 ml	75 ml
6.	Wash II Buffer*	7 ml	14 ml	35 ml
7.	Elution Buffer	10 ml	20 ml	50 ml
8.	Spin Column	50	100	250
9.	Collection Tubes	50	100	250

*Look at"Notes Before Starting" for preparation of Proteinase K and Wash Buffers.

†Precipitation may occur during storage. Heat to dissolve the precipitate formed before use.

Storage Conditions & Robustness

Kit components should be stored at room temperature (15-25°C) excluding Proteinase K. Do not expose Kit to direct sunlight. Proteinase K should be stored at -20 °C. When used under the seconditions, the kit can be stored for 12 months without any performance loss.

Intended Use

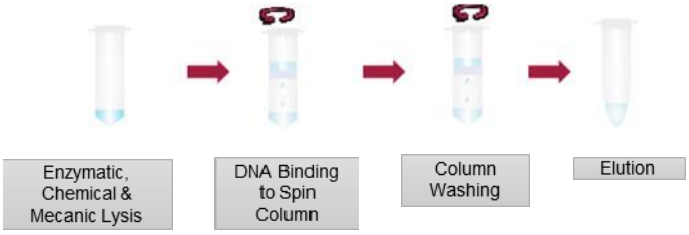
NucleoGene Stool DNA Quick Extraction Kit includes spin column and special lysis buffer system for effectively DNA extraction from stool samples. Specifically designed for DNA isolation and purification. Suitable for downstream applications such as purified DNA, PCR, Real Time Qualitative and Quantitative PCR (qPCR) and microarray. NucleoGene Stool DNA Quick Extraction Kit is designed for research use only. It is not for diagnosis and treatment of disease.

Product Usage Limits

- NucleoGene Stool DNA Quick Extraction Kit is for purification of nucleic acids of stool samples. It cannot be used for clinical purposes. Whether the kit is suitable for the user's own experimental design is up to the user's discretion.
- In particular, the ability of this kit to isolate DNA from Stool samples has been confirmed.
- The collection, transport and storage of the sample to be used are at least important as purification andare sensitive stages that may affect the results.
- It is designed for professional use by educated personnel only.
- Kits or any kit components with different lot numbers should not be used together.

Introduction

The NucleoGene Stool DNA Qu ck Extraction Kit provides quick and easy purification of total DNA from fresh or frozen stool samples. The purified DNA is of high quality and suitable for PCR and other downstream applications. With this kit, which has a simple procedure, it is possible to obtain pure DNA ready for direct use in a short time. Purification does not require phenol-chloroform extraction or precipitation with alcohol and requires minimal handling. DNA is separated by low salt content buffer and does not contain protein, nucleases and other impurities or inhibitors. Purified DNA is ready for use in PCR and other enzymatic reactions or can be stored at -20 ° C for later use. Stool samples typically contain many compounds that can degrade D A and inhibit downstreamenzymatic reactions. To ensure removal of these substances, NucleoGene Stool DNA Quick Extraction Kit contains Stool Buffer specially formulated to separate substances that restrict or make it impossible to use DNA from stool samples.



NucleoGene Stool DNA Quick Extraction Kit is optimized for use in 220 mg stool sample. Purified DNA with complete disolation should be used immediately or a freezer at -20 ° C should be preferred for long-term storage.

Warnings & Precautions

- All clinical specimens and residues and debris arising therefrom must be treated as potential infectious agents and disposed of accordingly.
- All samples should be prepared in Biosafety Level 1 and 2 or Class II Biosafety Cabins.
- All surfaces should be cleaned daily with a disposable paper towel or napkin by using freshly prepared Sodium Hypochlorite %20 Solution.
- Do not neglect to use laboratory safety consumable materials such as disposable gloves, goggles, visor, disposable cuffs, and disposable mask.
- If any of the kit components come into contact with your skin, wash it with plenty of water immediately. In case any of the kit components come into contact with your mucous membranes such as your eyes or mouth, wash the contact part with plenty of water again; and go to the doctor. If possible, prefer filtered pipette tips.
- Do not mix the kit solutions or waste appeared during isolation with bleach.
- Keep Kit out of contamination sources such as DNA and RNA, especially amplified nucleic acid.
- Do not mix solutions with different lot numbers or do not use or combine products from othe companies.
- For more information, please apply the Material Safety Data Sheet(MSDS), which you can request from www.nucleogene.com.

E-mail: i nfo@nucleogene.com
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- **Other Required Materials**

- Thermal Block or water bath
- Ethanol (%96-100)
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Mikrocentrifuge tubes (2.0 ml)
- Micropipette set and sterile filtered micropipette tips
- Spatula

Notes Before Starting

If the kit is kept in a non-air-conditioned environment, Lysis Buffer and Inhibitor Removal Buffer solution can precipitate at room temperature in cold weather. In such a case to dissolve the precipitate, the Lysis buffer and Inhibitor Removal Buffer solution is held at 50°C in a water bath until dissolving completely. Be sure of all solutions are at the room temperature before and after protocol implementa- tion, if they are not, allow them to reach room temperature.

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

Wash Buffer II
Add 23 mL of absolute or 96 % ethanol for 50 Test
Add 56 mL of absolute or 96 % ethanol for 100 Test
Add 115 mL of absolute or 96 % ethanol for 250 Test

Then, do not forget shaking with your hand or with vortex at least 10 seconds.

Proteinase K is supplied as lyophilizing. Before starting to use the kit, all lyophilized Proteinase K should be mixed with Pro- teinase K buffer (Add 1000 µl Proteinase K Buffer for per vial for 50 and 100 Tests. Add 5000 µl Proteinase K Buffer for per vial for 250 Tests.)

Protocol

Control the reagents

Before starting to DNA isolation, control reagents that are free of leakage. To mix the solutions, shake the reagents slightly. If rea- gents precipitate (specially Lysis Buffer), they dissolve at 50 ° C by heating.

Stool DNA Extraction

1. Weigh 220 mg of feces in a 2 ml microcentrifuge tube (not supplied) and place the tube on ice.

2. Add 1 ml of Inhibitor Removal Buffer to each stool sample. Vortex continuously for 1 minute or until the stool sample is thoroughly homogeneous.

3. Heat the suspension at 95° C for 10 minutes. In this step, vortex should be done every 1 minute or the process should be carried out in a dry block with shaking heater.

4. Centrifuge the sample at max speed (minimum 14,000 x g, do not exceed 20,000 x g) for 3 minutes to pellet the fecal particles.

IMPORTANT: Do not transfer any particles to the new tube. If particles still appear in the supernatant, centrifuge the sample again.

5. Pipette 20 µl of proteinase K into a new 1.5 ml microcentrifuge tube (not supplied).

6. Pipette 200 µl of the supernatant from step 4 into a 1.5 ml microcentrifuge tube containing proteinase K.

7. Add 200 µl Lysis Buffer and vortex for 15 seconds.

Note: Do not add Proteinase K directly to the Lysis Buffer. It is important to mix the sample and Lysis Buffer thoroughly to form a homogeneous solution.

8. Incubate at 70° C for 15 minutes. Spin centrifuge briefly to remove any drops inside the tube cap (optional).

9. Add 200 µl of ethanol (96 – 100%) to the lysate and mix by vortexing. Spin centrifuge briefly to remove any drops inside the tube cap (optional).

10. Carefully transfer 600 µl of the lysate from step 9 to the Spin column. Close the lid and centrifuge for 1 minute at max speed (minimum 14,000 x g, do not exceed 20,000 x g). Pour off the liquid in the collection tube and place the Spin column in the collection tube. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the spin column is empty.

11. Carefully open the spin column and add 500 µl Wash I. Centrifuge at max speed (minimum 14,000 x g, do not exceed 20,000 x g) for 1 minute. Pour off the liquid from the collection tube and place the Spin column back into the collection tube.

12. Carefully open the spin column and add 600 µl of Wash II. Centrifuge at max speed (minimum 14,000 x g, do not exceed 20,000 x g) for 1 minute. Pour off the liquid from the collection tube and place the Spin column back into the collection tube.

13. Centrifuge the spin column at maximum speed (minimum 14,000 x g, do not exceed 20,000 x g) for 2 minutes to remove the remaining ethanol. Discard the collection tube and place the Spin column in a new 1.5 ml microcentrifuge tube.

14. Pipette 100 µl of Elution Buffer directly into the middle of the spin column. Incubate for 1 minute at room temperature, then centrifuge at 14,000xg for 1 minute to obtain DNA.

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Troubleshooting

Issue	Reason	Solution
Clogged Column	Overuse sample	If more than 220 mg of sample is used, increase the Proteinase K, Solution volumes by the same rate. Pass the lysate through a column.
	Incompleted lysis	Be sure for mixing well of sample and lysis solution.
Low DNA amount	Wrong washing	Verify that the Buffer 6-7 solution concentrates are diluted with the specified volumes of ethanol. To prevent evaporation, close the caps of the bottles thoroughly.
	Weak elution	Reduce Elution volume.
	Clogged column	Lookup
Enzymatic reactions do not run with isolated DNA.	Low DNA concentration	Precipitate DNA with ethanol and then dissolved with low volume of Elution Buffer.
	High salt content of isolated DNA	Precipitate DNA with ethanol.
	Ethanol remaining from washing buffer	After washing stage, centrifuge the spin column to remove remaining ethanol.

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