NucleoGene
Saliva DNA Extraction Kit
Instructions for Use

Release Date— 01.12.2023

REF

NGE038– 50 Test
100 Test
250 Test

For the isolation of Saliva 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†	25 ml	50 ml	125 ml
2.	Wash Buffer I*	15 ml	30 ml	75 ml
3.	Wash Buffer II*	15 ml	30 ml	38x2 ml
4.	Elution Buffer	10 ml	20 ml	75 ml
5.	Proteinase K*	1 vial	2 vial	5 vial
6.	Spin Columns	50	100	250
7.	Collection Tubes	50	100	250
8.	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). Proteinase K should be stored at -20 ° 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

The NucleoGene Saliva DNA Extraction Kit specifically designed for the efficient purification different types of starting materials like saliva samples up to 250 ul. Samples DNA is bound to the filter in the spin column and is released using a special buffer system. The NucleoGene Saliva DNA Extraction Kit can be easily adapted to isolation devices and workstations using automatic spin columns. Thanks to the easy and fast working procedure of the kit, different types samples DNA can be purified from several samples simultaneously with a simple laboratory infrastructure. Purified DNA is suitable for various procedures such asPCR, qPCR, Digital PCR, NGS, Microarray, etc.

Product Usage Limits

- The NucleoGene Saliva 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 saliva samples has been confirmed.

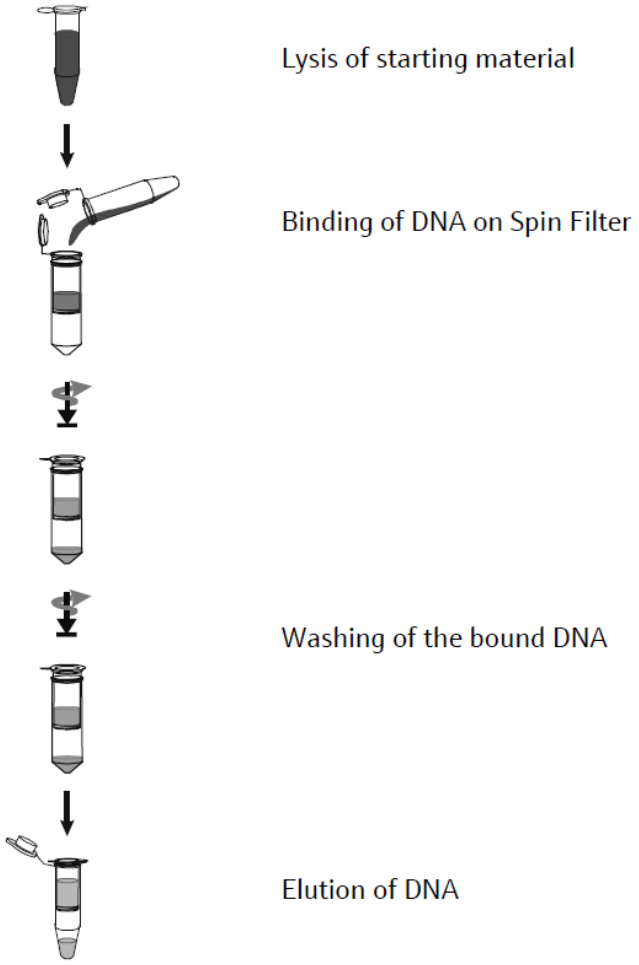
- Picking, transporting and storing the samples to be used is at least as sensitive as the purification and can be sensitive to the result.

- Designed for professional use only by trained personnel.

- Kit components with different lots should not be combined used together.

Introduction

NucleoGene Saliva DNA Extraction Kit provides a fast, simple and cost-effective method for the isolation of DNA from saliva 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 Saliva 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
- Thermal block or water bath
- 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 Saliva Collection and Preservation Kit); optional

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

Proteinase K:
Add 1000 µl Nuclease Free Water for per vial

Store lyophilized Proteinase K at 4 ° C to 8 ° C. Divide dissolved Proteinase K into aliquots and store at -22 ° C to -18 ° C. Repeated freezingand thawing will reduce the activity dramatically.

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.

Saliva DNA Extraction

1. Prior to collection of saliva samples, the donor must rinse their mouth with a few milliliters of water for 10 seconds to remove any food particles that may be present. If food particles are present, they can cause blockage of the colon.

2. Ten minutes after rinsing, collect saliva by spitting into a sterile collection tube or bottle (not supplied). The amount of saliva collected should be at least 100 uL and not more than 2 mL.

3. Transfer 250 µL of liquid saliva into a sterile microcentrifuge tube.

4. Add 20 µL of Proteinase K (vortex before using Proteinase K). Mix by vortexing.

5. Add 250 µL Lysis Buffer and mix by vortexing and incubate at 56° C for 15 minutes.

6. Add 250 µL Absolute Ethanol to the saliva sample. Mix by vortexing and incubate for 2 minutes at room temperature.

7. Place the spin column in the collection tubes provided.

8. Add up to 770 µL of lysate to the column and centrifuge at 4000 x g for 1 minute.

Note: Inspect the column to ensure that all of the sample passes into the collection tube. If the entire sample volume has not passed, centrifuge for an additional 1 minute.

9. Discard any remaining liquid and reinsert the spin column into the collection tube.

Note: Each spin column provided is capable of handling up to 600 µL of lysed saliva. If additional DNA isolation is desired, use an additional spin column. For example, if 1.2 mL of saliva is to be run, use two spin columns and run 600 µL of fractionated saliva with each column.

10. Repeat until all the lysate has passed through the column.

11. 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.

12. 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

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

14. Place the spin column in a clean 1.5 mL microcentrifuge tube.

15. Add 50-100 µL of Elution Buffer pre-warmed at 70° C to the spin column. Incubate for 5 minutes at room temperature then centrifuge for 2 minutes at 8000 x g.

16. 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 pellet un-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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