NucleoGene
Cell Free DNA Extraction Kit
Instructions for Use

Release Date— 01.12.2023

REF

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

NGE034– 50 Test
100 Test
250 Test

Kit Contents

	Material Supplied	50 Test	100 Test	250 Test
1.	Lysis Buffer†	40 ml	80 ml	200 ml
2.	Binding Buffer	20 ml	40 ml	100 ml
3.	Wash Buffer I*	18 ml	36 ml	90 ml
4.	Wash Buffer II*	12 ml	24 ml	30x2 ml
5.	Elution Buffer	10 ml	20 ml	75 ml
6.	Carrier Mix	1 vial	1 vial	1 vial
7.	Proteinase K*	1 vial	2 vial	5 vial
8.	Proteinase K Buffer	1 vial	2 vial	5 vial
9.	Spin Columns	50	100	250
10	Collection Tubes	50	100	250
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* 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 and Carrier Mix 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 Cell Free DNA Extraction Kit specifically designed for the efficient purification different types of starting materials like serum or plasma samples up to 600 ul. Samples DNA is bound to the filter in the spin column and is released using a special buffer system. The NucleoGene Cell Free 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.

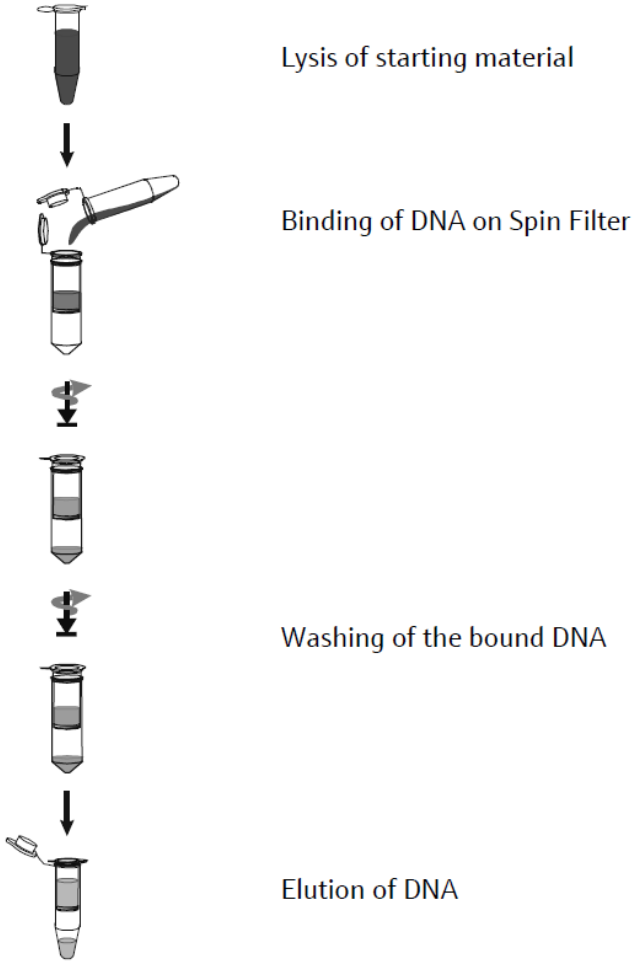
Free-circulating nucleic acids, such as tumor-specific extracellular Nucleic Acid fragments and mRNAs in the blood or fetal nucleic acids in maternal blood, are present in serum or plasma usually as short fragments, <1000bp(Nucleic Acid). The NucleoGene Cell Free DNA Extraction Kit enables efficient purification of these circulating nucleic acids from human plasma, serum, or urine. Samples can be either fresh or frozen (provided that they have not been frozen and thawed more than once). Free-circulating cell-free DNA or viral nucleic acids are eluted in Elution Buffer, ready for use in amplification reactions or storage at －30 to －15° C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

Product Usage Limits

- The NucleoGene Cell Free DNA Extraction Kit intended for purification of DNA from samples such as serum, plasma or urine. 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 serum, plasma or urine 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

The NucleoGene Cell Free DNA Extraction Kit provides a fast, simple and cost-effective method for the isolation of DNA from serum, plasma or urine 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. The NucleoGene Cell Free DNA Extraction Kit suitable for a variety of routine applications including isolatedDNA, Real-Time PCR, PCR, DNA sequence analysis, NGS, Digital PCR, 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.

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- 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 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

• Isopropanol

•Microcentrifuge

• Vortex mixer

• Microcentrifuge tubes (1.5 ml)

• Microcentrifuge tubes (2.0 ml)

• Micropipette set and micropipette tips with sterile filter

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 12 mL of 96 % or absolute ethanol for 50 tests
Add 24 mL of 96 % or absolute ethanol for 100 tests
Add 60 mL of 96 % or absolute ethanol for 250 tests

Wash Buffer II:
Add 48 mL of 96 % or absolute ethanol for 50 tests
Add 96 mL of 96 % or absolute ethanol for 100 tests
Add 120 mL of 96 % or absolute ethanol per bottle for 250 tests

Proteinase K:
Add 1000 µl Proteinase K Buffer 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.

Carrier Mix:
Add 250 ul of Nuclease Free Water for 50 tests
Add 500 ul of Nuclease Free Water for for 100 tests
Add 1250 ul of Nuclease Free Water for for 250 tests

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

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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.

Cell Free DNA Extraction

1. Pipet 20 µl Proteinase K into a 2ml centrifuge tube.

2. Add 0.6 ml of serum or plasma to the tube, mix thoroughly.
To obtain cell-free nucleic acids from urine or plasma, it is recommended to centrifuge the samples at high speed (e.g., 16,000 x g) for 10 min at 4°C and only use the supernatant for nucleic acid extraction. This will remove cellular material and cellular nucleic acids from the sample.

3. Add 0.6 ml Buffer Lysis Buffer and 5µl of Carrier Mix to the tube, Close the cap and mix thoroughly by pulse-vortexing for 15s. If the sample volume is larger than 600µl, increase the amount of Proteinase K and Lysis Buffer proportionally. Do not add Proteinase K directly to Lysis Buffer. Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.

4. Incubate at 55°C for 15min.

5. Add 300 ul Isopropanol to the lysate in the tube, Close the cap and mix thoroughly by pulse-vortexing for 30s. Incubate for 3min at room temperature.

6. Insert a Spin Column in a 2ml Collection Tube.

7. Add up to 750µl solution from Step 5 to the Column. Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

8. Repeat Step 7 until all of the sample has been transferred to the column. Discard the filtrate and reuse collection tube.

9. Insert the column in a new 2ml Collection Tube. Add 600µl Buffer Wash Buffer I to the column. Centrifuge at 12,000 × g for 1 minute.Discard the filtrate and reuse collection tube.

10. Add 600µl Buffer Wash Buffer II to the column. Centrifuge at 12,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.

11. Repeat Steps 10 for a second Wash Buffer II wash step.

12. Centrifuge the empty column at 12,000 × g for 2 minute at room temperature to dry the column matrix.

13. Place the column to a clean 1.5ml microcentrifuge tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

14. Add 20-100µl Elution Buffer directly to the center of the column membrane.Let sit at room temperature for 3 minutes. Centrifuge at 12,000 × g for 1 minute at room temperature. Store Nucleic Acid at -20°C.

Ensure that Elution Buffer is equilibrated to room temperature. If elution is done in small volumes (<50µl), the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA. Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will be up to 5µl less than the elution volume applied to the Mini column.

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.
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.

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