NucleoGene
Genomic DNA Extraction Kit
Instructions for Use

Release Date— 01.06.2023

CE IVD

REF

For the isolation of Blood,Tissue and Cell DNA from samples.
For nucleic acid isolation and purification.
For research purposes only.
Not suitable for diagnostic use.

NGE040– 50 Test
100 Test
250 Test

Kit Contents

	Material Supplied	50 Test	100 Test	250 Test
1.	Tissue-Cell Lysis Buffer†	10 ml	20 ml	50 ml
2.	Blood Lysis Buffer	10 ml	20 ml	50 ml
3.	Binding Buffer	15 ml	30 ml	75 ml
4.	Wash Buffer I*	13 ml	26 ml	90 ml
5.	Wash Buffer II*	15 ml	30 ml	38x2 ml
6.	NES Buffer	0,5 ml	1 ml	2,5 ml
7.	DTT Buffer*	1 vial	2 vial	5 vial
8.	Melt Buffer	15 ml	30 ml	75 ml
9.	Elution Buffer	10 ml	20 ml	75 ml
10.	Proteinase K*	1 vial	2 vial	1 vial
11.	Spin Columns	50	100	250
12.	Collection Tubes	50	100	250
13.	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

The NucleoGene Genomic DNA Blood,Tissue & Cell Isolation Kit specifically designed for the efficient purification different types of starting materials like blood, tissue samples up to 50 mg, paraffin-embedded tissue material,buccal swabs, mouse or rodent tail, eukaryotic cell pellets, saliva and etc. Samples DNA is bound to the filter in the spin column and is released using a special buffer system. The NucleoGene Genomic DNA Tissue & Cell Isolation 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 infra structure. Purified DNA is suitable for various procedures such asPCR, qPCR, Digital PCR, Microarray, etc.

Product Usage Limits

- The NucleoGeneGenomic DNA Blood Tissue & Cell Isolation Kits intended for purification of DNA from samples such as different typessamples tissue and cell. 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 different types samples tissue and cell 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.

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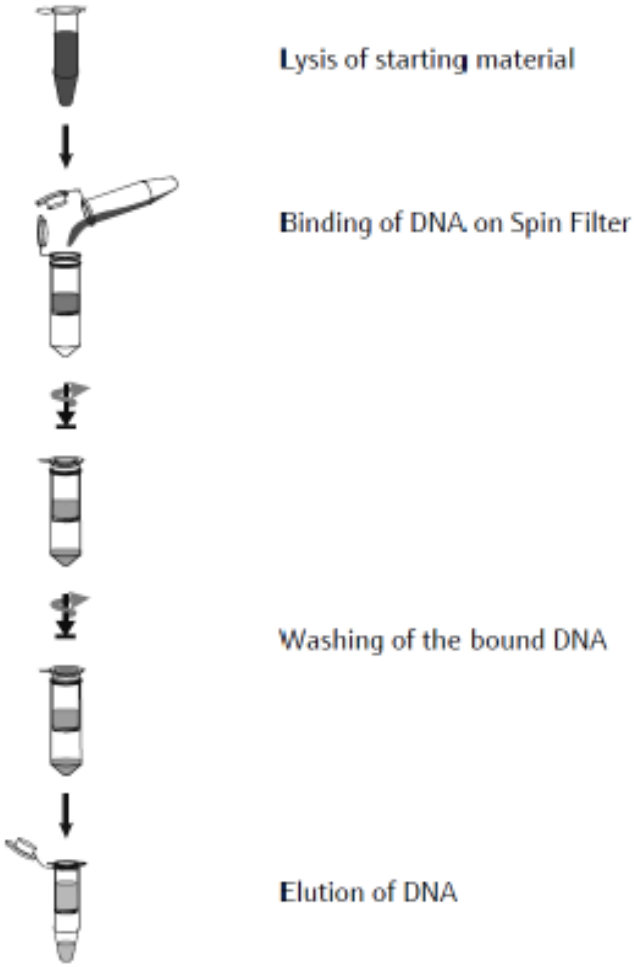
Introduction

NucleoGene Genomic DNA Blood,Tissue & Cell Isolation Kit provides a fast, simple and cost-effective method for the isolation of DNA from different types of 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 macro molecular 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 Genomic Blood DNA Tissue & Cell DNA Isolation Kitis suitable for a variety of routine applications including isolated DNA, Real-Time PCR, PCR, DNA sequence analysis, 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.
- 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 n 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 t xic 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
- RNase A (10 mg/ml); optional
- Xylene
- Lyticase (0,5 mg/ml)
- Lysozyme (10mg/ml
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Microcentrifuge tubes (2.0 ml)
- Micropipette set and micropipette tips with sterile filter
- Spatula

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 13 mL of 96 % or absolute ethanol for 50 tests
Add 26 mL of 96 % or absolute ethanol for 100 tests
Add 65 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 Proteinase K Bufferfor per vial for 50 and 100 Tests
Add 5000 µl Proteinase K Buffer for per vial for 250 Tests

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.

DTT Buffer is supplied as lyophilizing. Before starting to use the kit, all lyophilized DTT Buffer should be mixed with 800 ul of Nuclease Free Water.

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.

Blood DNA Isolation

Before starting Blood 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.

IMPORTANT

Pre-fill the needed amount of **Elution Buffer** into a 2.0 ml reaction tube and incubate the **Elution Buffer** at 60 °C until the elution step. If the sample volume is less than 100-200 µl, add the appropriate volume of PBS.

1. Add 20 µl of Proteinase K to a 2 ml tube and add 200 µl of Whole Blood sample on top of it and vortex. Add 200 µl of Blood Lysis Buffer to the mixture and vortex again for 30 seconds, then incubate for 12 minutes at 56° C. After incubation, add 200 µl of Absolute Ethanol to the tube, cap the tube and vortex for 30 seconds.

NOTE
We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

The kit co-purify DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 1-2 µl of a RNase A stock solution (10 mg/ml) to the sample before addition of Isopropil alchol, vortex shortly and incubate for 5 minutes at room temperature.

1. Place the spin column in the Collection tube and mixture is transferred to the spin column. Centrifuge at 8,000 xg for 1 min. Discard the liquid portion from the collection tube and place the spin column back into the collection tube.

2. 500 µl of Wash Buffer I is added to the spin column. Centrifuge at 8,000 xg for 1 min. Discard the liquid portion from the collection tube and place the spin column back into the collection tube.

3. Add 700 µl of Wash Buffer II to the spin column. Centrifuge at 14,000 xg for 1 min to remove all traces of ethanol. Discard the Collection Tube.

4. Place the Spin column into an 1.5 ml microcentrifuge tube. Carefully open the cap of the Spin Filter and add 50-200 µl Elution Buffer (pre-warmed at 60 °C). Incubate at room temperature for 2 minutes.

5. Centrifuge at 8,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of pre-warmed Elution Buffer (e.g. 100 µl + 100 µl) might increase the yield of extracted gDNA.

6. The received liquid contains DNA and is ready to use. For long-term storage, the DNA is stored at -20°C.

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Tissue DNA Isolation

7. The 50 mg of tissue sampleinto small pieces and place the tissue in 2 ml tube and vortex by adding 200 µl of Tissue-Cell Lysis Buffer and 20µl of Proteinase K and then incubate for until the sampleis completely lysed at 56 ° C(approx. 1-2 hours; especially for rodent tails usenot more than 2 hours for lysis).

8. After incubation, centrifuge the 2 ml tube at 10,000 x g for1 minuteto spin down unlysed material. Transfer the supernatantinto another 1.5 ml tube.

OPTIONAL;To remove RNA from the sample (if necessary) add 2 µl of RNase A solution (10 mg/ml), vortex shortly and incubate for 5 minute at RT.

9. Add 225 µl of Binding Buffer and 225 µl Absolute Ethanol is added to the tube and with vortex is mixed for 30 seconds.

10. All mixture is transferred to the spin column. Centrifuge at 10,000 x g for 2 min. The liquid in the bottom tube is discarded and the column is placed in a new collection tube.

11. 500 µl of Wash Buffer I is added to the spin column. Centrifuge for 1 minute at 10,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a new collection tube.

12. Add 750 µl of Wash Buffer II to the spin column. Centrifuge for 1 minute at 10,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a new collection tube. Optional this operation is performed 2 times.

13. The spin column is centrifuged at 10,000 x g for 3 min to remove residual ethanol.

14. The spin column is transferred to a sterile 1.5 ml microcentrifuge tube. Add 50-200µl of Elution Buffer to the center of the spin column and incubate for 1 minutes at room temperature.

15. The spin column is centrifuged at 10,000 x g for 1 minute. The received liquid contains DNA and is ready to use. For long-term storage, the DNA is stored at -20°C.

Cell DNA Isolation

1. The 5 x 10^6cells(bacteria, mammalian culture, yeast etc.) in 2 ml tube by centrifugation for 10 minutes at 5,000 xg and discard supernatant. Adding 200 µl of Tissue-Cell Lysis Buffer, 20µl of Proteinase K, 20 µl Lysozyme for bacteria and 40 µl Lyticase for yeast then incubate for until the sampleis completely lysed at 56 ° C.

2. After incubation,Add 225 µl of Binding Buffer and 225 µl Absolute Ethanol is added to the tube and with vortex is mixed for 30 seconds.

3. All mixture is transferred to the spin column. Centrifuge at 10,000 x g for 2 min. The liquid in the bottom tube is discarded and the column is placed in a new collection tube.

4. 500 µl of Wash Buffer I is added to the spin column. Centrifuge for 1 minute at 10,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a new collection tube.

5. Add 750 µl of Wash Buffer II to the spin column. Centrifuge for 1 minute at 10,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a new collection tube. Optional this operation is performed 2 times.

6. The spin column is centrifuged at 10,000 x g for 3 min to remove residual ethanol.

7. The spin column is transferred to a sterile 1.5 ml microcentrifuge tube. Add 200 µl of Elution Buffer to the center of the spin column and incubate for 1 minutes at room temperature.

8. The spin column is centrifuged at 10,000 x g for 1 minute. The received liquid contains DNA and is ready to use. For long-term storage, the DNA is stored at -20°C.

Paraffin Embedded Tissue Samples DNA Isolation

Deparaffinization with NucleoGene Melt Buffer

1. Using a microtome or scalpel,

Tissue Type	Thickness	Amount
Surgical FFPE tissue scrolls	5~8 µm (5 µm is recommended)	4~6 scrolls (5 scrolls are recommended)
Biopsy FFPE tissue scrolls	5~8 µm (5 µm is recommended)	8~12 scrolls (10 scrolls are recommended)
FFPE tissue slides	/	6~8 slides (7 slides are recommended)

2. Transfer the sections to a 1.5 mL centrifuge tube.

3. Add 300 µl of Melt Buffer to it. Incubate for 20 minutes at 90 °C.

4. When incubation is finished, centrifuge the tubes at maximum speed for 2 minutes and aspirate all liquid. After this step, the isolation phase can be started or deparaffinization can be continued.

Deparaffinization with Ethanol and Xylene

1. Add 1 mL of xylene to the tissues treated with Melt Buffer, close the lid and vortex vigorously for 10 seconds. Centrifuge at maximum speed (~ 18000 xg) for 2 minutes at room temperature.
2. Pipet and discard the supeDNAtant (do not remove any pieces from the pellet).
3. Add 1 mL of ethanol (96% ~ 100%) to the pellet and mix by vortexing for 10 seconds.
4. Centrifuge for 2 minutes at room temperature at maximum speed.
5. Pipette out the supernatant (do not remove any of the pellets).
6. Uncap the tubes and incubate for 10 minutes at room temperature or 5 minutes at 37°C.
Make sure any remaining ethanol has evaporated before continuing.

FFPE DNA Isolation

1. Add 180 µL of Tissue-Cell Lysis Buffer, 14 µL DTT Buffer and 20 µL of Proteinase K solution to the remaining precipitates at the bottom of the tube and incubate (with shaking) for 60 min at 56 °C or need higher concentration of DNA, incubate for further time or overnight.

2. After incubation, 10 µl of NES Buffer is added and incubation is continued at 90°C for 2 hours.

2. After incubation, immediately centrifuge for 1 minute at maximum speed to separate the tissue lysate from the paraffin and allow the paraffin to form a thin layer on the top.

3. Using a clean, DNAse-free pipette tip (1 ml), penetrate the paraffin layer and aspirate the clear solution containing tissue lysate into a clean DNAse-free tube.

Note: The opening of the pipette tip can be cut using a clean DNAse-free cutting material to more easily transfer the lysate solution to the new tube in case the tip becomes clogged with paraffin.

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4. Transfer the solution DNase Free Tube add 200 µL Binding Buffer and 200 µL absolute ethanol to above mixture, and vortex 5 s. Transfer the solution obtained from step to a spin column (DNA Absorption Column) with a collection tube; centrifuge at 8,000 g for 30 s; discard the waste in the collection tube; and place the column back in the collection tube.

5. Add 600 µL of Wash Buffer I to the DNA Spin Column, centrifuge at 8000 xg for 30 s.

6. Discard the liquid in the collection tube and place the spin column back in the collection tube.

7. Add 600 µL of Wash Buffer II to the DNA Spin Column, centrifuge at 8000 xg for 30 s.

8. Discard the liquid in the collection tube and place the spin column back in the collection tube, centrifuge for 1 minute at 14000 xg.

9. Discard the collection tube containing the liquid and place the DNA Spin Column in a clean 1.5 mL centrifuge tube.

10. Transfer 30 ~ 80 µL of Elution Buffer pre-warmed at 65 °C to the center of the filter of the DNA Spin Column and incubate for 3 minutes at room temperature. Be careful not to touch the pipette tip to the filtered part of the column.

14. After incubation, centrifuge the tubes for 1 minute at 8000 xg.

15. The liquid remaining in the tube contains DNA and is ready to use. For long-term storage, DNA is stored at -20 °C.

Buccal Swab DNA Isolation

1. Place the swab into a 1.5 ml reaction tube and vortex by adding 200 µl of Lysis Buffer and 20 µl of Proteinase K and then incubate for until the sampleis completely lysed at 56 ° C 15 min.

2. After lysis time remove the swab from the tube and squeeze theswab on the wall of the tube to remove all Lysis Buffer fromthe swab.

3. Add 225 µl of Binding Buffer and 225 µl Absolute Ethanol is added to the tube and with vortex is mixed for 30 seconds.

4. All mixture is transferred to the spin column. Centrifuge at 10,000 x g for 2 min. The liquid in the bottom tube is discarded and the column is placed in a new collection tube.

5. 500 µl of Wash Buffer I is added to the spin column. Centrifuge for 1 minute at 10,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a new collection tube.

6. Add 750 µl of Wash Buffer II to the spin column. Centrifuge for 1 minute at 10,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a new collection tube. Optional this operation is performed 2 times.

7. The spin column is centrifuged at 10,000 x g for 3 min to remove residual ethanol.

8. The spin column is transferred to a sterile 1.5 ml microcentrifuge tube. Add 200 µl of Elution Buffer to the center of the spin column and incubate for 1 minutes at room temperature.

9. The spin column is centrifuged at 10,000 x g for 1 minute. The received liquid contains DNA and is ready to use. For long-term storage, the DNA is stored at -20°C.

Saliva DNA Extraction Protocol

Fresh Saliva Samples

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. Spin may cause blockage of the colon if food particles are present.

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 250 µL Blood Lysis Buffer and mix by vortexing.

5. Add 20 µL of Proteinase K (vortex before using Proteinase K). Mix by vortexing and incubate at 56° C for 15 minutes.

6. Add 200 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 760 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 Biffer 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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