NucleoGene Blood Tissue Cell DNA Extraction Kit

Instructions for Use

Release Date— 01.12.2022

REF

NGE033– 50 Test
100 Test
250 Test

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.

Kit Contents

	Material Supplied	50 Test	100 Test	250 Test
1.	Tissue-Cell Lysis Buffer†	20 ml	40 ml	100 ml
2.	Blood Lysis Buffer	10 ml	20 ml	50 ml
3.	Binding Buffer	20 ml	40 ml	100 ml
4.	Wash Buffer I*	13 ml	26 ml	2x45 ml
5.	Wash Buffer II*	15 ml	2x15 ml	19x4 ml
6.	Elution Buffer	10 ml	20 ml	75 ml
7.	Proteinase K*	1 vial	2 vial	1 vial
8.	Spin Columns	50	100	250
9.	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). 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

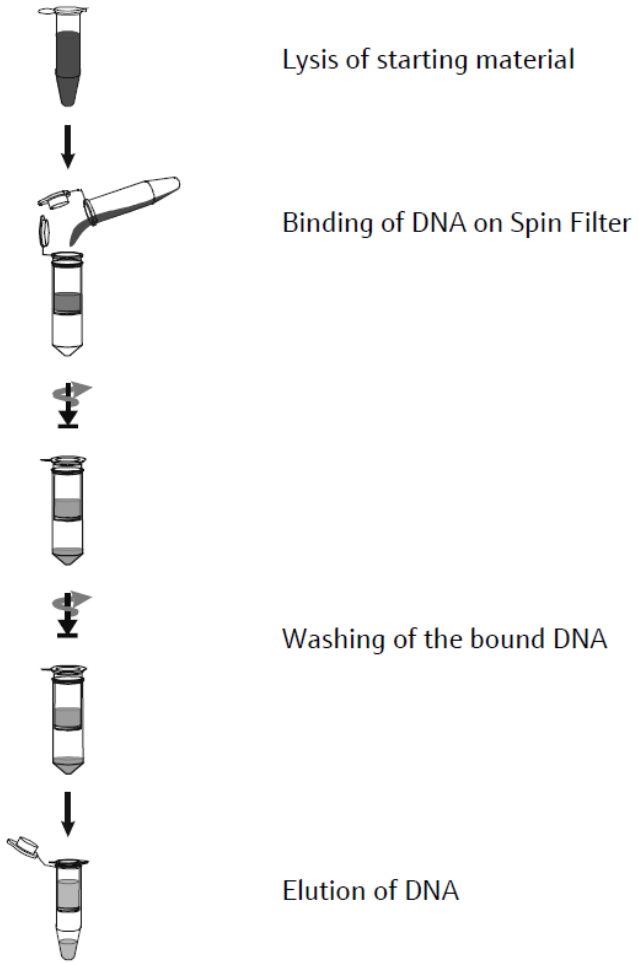
The NucleoGene Genomic DNA Blood,Tissue & Cell Isolation Kit specifically designed for the efficient purification different types of starting materials like blood, tissue samples up to 50 mg, paraffin-embedded tissue material,buccal swabs, mouse or rodent tail, eukaryotic cell pellets and etc. Samples 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.

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 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.

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- 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
•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 33 mL of 96 % or absolute ethanol per bottle for 250 tests

Wash Buffer II:
Add 60 mL of 96 % or absolute ethanol for 50 tests
Add 60 mL of 96 % or absolute ethanol per bottle for 100 tests
Add 75 mL of 96 % or absolute ethanol per bottle for 250 tests

Proteinase K:
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

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.

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 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 400 µl of 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 400 µl of Binding Buffer is added to the tube and with vortex is mixed for 15 seconds.

10. 800µl of the 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 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 400 µl of 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, 400 µl of Binding Buffer is added to the tube and with vortex is mixed for 15 seconds.

3. 800 µl of the 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

1. Using a microtome or scalpel, cut 3-8 segments with a thickness of 5 ~ 10 µm and a surface area of 0.5 ~ 1 cm^2. Transfer the sample to a 2 ml tube and add 1 ml Xylene onto it.Follow thedissolution until the tissue sample looks transparent (while paraffinremains white).

2. Centrifuge at maximum speed for 5 minutes at room temperature. Discard the supernatant very carefully by aspirating with a pipette.

3. Add 1 ml ethanol (96–99.8 %) to the pellet and vortex vigorously. Centrifuge at maximum speed at room temperature for 3 minutes and remove the ethanol by pipetting. Repeat the washing step with ethanol once again.

4. Incubate the open tube at 37 °C for 10–15 minutes to evaporate the residual ethanol completely.

NOTE: If you want a better result, you should use Nucleogene Melt Buffer. Using Nucleogene Melt Buffer; Using a microtome or scalpel, cut 3-8 segments with a thickness of 5 ~ 10 µm and a surface area of 0.5 ~ 1 cm2. Transfer the sample to a 2 ml tube and add 300 µl of Nucleogene Melt Buffer and incubate at 90°C for 20 minutes. After incubation, carefully pull out all of the Nucleogene Melt Buffer without removing the tissues, for this you can centrifuge the tube at 11,000xg for 3 minutes and carefully pull the Nucleogene Melt Buffer away with a pipette. After these procedures, you can proceed to the Lysis stage.

5. Adding 400 µl of Lysis Buffer and 20 µl of Proteinase K then incubate for until the sampleis completely lysed at 56 ° C.

6. Pre-heat the thermal mixer without the sample to 90 °C, afterwards incubate the lysed sample for 60 minutes at 90 °C.

7. After incubation, 400 µl of Binding Buffer is added to the tube and with vortex is mixed for 15 seconds.

8. 800 µl of the 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.

9. 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.

10. 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.

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

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

13. 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.

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Buccal Swab DNA Isolation

1. Place the swab into a 1.5 ml reaction tube and vortex by adding 400 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 400 µl of Binding Buffer is added to the tube and with vortex is mixed for 15 seconds.

4. 800 µl of the 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.

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. Overloading 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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