NucleoGene
Blood DNA Extraction Kit
Instructions for Use

Release Date— 01.02.2021

REF

For the isolation of fresh or frozen Whole Blood DNA from

samples.
For nucleic acid isolation and purification.

NGE005– 50 Test
100 Test
250 Test

For research purposes only.

Not suitable for diagnostic use.

Kit Contents

	Material Supplied	50 Test	100 Test	250 Test
1	Lysis Buffer†	10 ml	20 ml	50 ml
2	Wash Buffer I *	15 ml	30 ml	75 ml
3	Wash Buffer II *	14 ml	28 ml	35x2 ml
4	Elution Buffer	10 ml	20 ml	50 ml
5	Proteinase K	1 vial	2 vial	1 vial
6	Proteinase K Buffer	1 ml	2 ml	5 ml
7	Spin Columns	50	100	250
8	Collection Tubes	50	100	250
9	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 NucleoGeneBlood DNA Isolation Kitis specifically designed for the efficient purification of mammalian samples. Mammalian blood samples DNA is bound to the filter in the spin column and is re- leased using a special buffer system. The NucleoGeneBlood DNA Isolation Kit can be easily adapted to isolation devices and work- stations using automatic spin columns. Thanks to the easy and fast (20 minute) working procedure of the kit, mammalian blood 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, Microarray, etc.

Product Usage Limits

- The NucleoGeneBlood DNA Isolation Kitis intended for purification of DNA from samples such as mammalian blood. 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 mammalian blood sampleshas 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

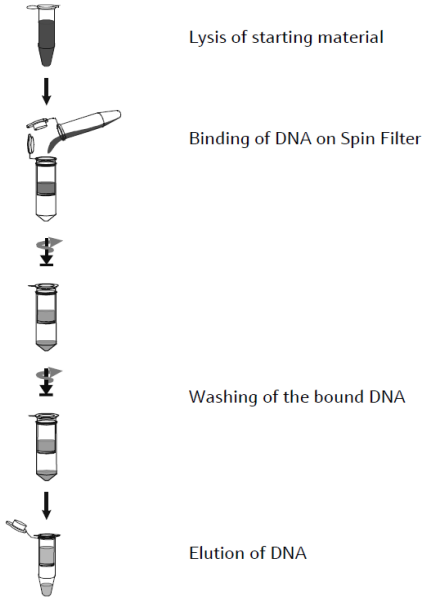
The NucleoGene Blood DNA Extraction Kit is designed as a very effective tool for rapid isolation of genomic DNA from whole blood samples for later in vitro diagnostic purposes. The kit can be used for genomic DNA isolation from fresh or frozen blood; Stabilized with EDTA or citrate from common blood collection systems. Sample volumes from 200 µl can be processed. If using smaller volumes of blood, apply sterile PBS up to 200 µl final sample volume.

The extraction procedure is based on a new chemistry and combines lysis of the blood sample with subsequent binding of nucleic acids to the surface of the Spin Filter membrane. After several washing steps, nucleic acids are separated from the membrane using Elution Buffer. The extraction chemistry and extraction protocol has been optimized to achieve maximum yield.

The NucleoGene Blood DNA Extraction Kit is not for use with cell- free body fluids such as cerebrospinal fluid, serum, plasma or urine, tissue or stool samples. Kit performance has not been evaluated with buffy coat, cultured or isolated cells, swabs, dried blood stains and viral DNA. The kit is also not specified for the isolation and purification of fungal, bacterial or parasitic nucleic acids.

The kit is intended for use by professional users. The kit is designed to be used for a wide variety of downstream applications such as amplification reactions and other analytical procedures. Diagnostic results generated using the extraction procedure in conjunction with diagnostic tests should be interpreted in relation to other clinical or laboratory results. Internal controls should be used for downstream applications to reduce irregularities in diagnostic results.

The NucleoGene Blood DNA Extraction Kit does not provide a diagnostic result. The use and validation of the kit in conjunction with the downstream in vitro diagnostic test is the sole responsibility of the user. The whole procedure can be completed in 20 minutes.



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 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
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 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 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 II: Add 56 mL of 96 % or absolute ethanol for 50 tests
Add 112 mL of 96 % or absolute ethanol for 100 tests
Add 140 mL per bottle of 96 % or absolute ethanol 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

Protocol

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 5 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.

2. 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.

3. 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.

4. Add 700 µl of Wash Buffer II 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.

5. Add 700 µl of Wash Buffer II 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.

6. Centrifuge the spin column for 2 minutes at 14,000xg to remove any remaining ethanol. Discard the Collection Tube after centrifuga- tion.

7. 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.

8. 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.

9. 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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Troubleshooting Guide

Problem	Reason	Solution
Poor or Low DNA Recovery	DNase contamination	Provide an DNase-free working environment. For long-term storage freeze the DNA eluate at –20°C.
	Improper washing	Confirm the wash solution concentrates were diluted with the specified volumes of ethanol. Keep bottles tightly capped between uses to prevent evaporation.
	Poor Elution	Repeat the elution step or increase the elution volume.
	Degradation or low concentration of Enhancer	Confirm the concentration and storage temperature of the dissolved lyophilized Enhancer aliquots. Do not freeze-thaw one aliquot over 3-4 times.
Downstream applications using recovered DNA do not proceed	DNA concentration is too low	Precipitate DNA with alcohol, and then resuspend DNA in a smaller volume of Solution Elution or dH2O.
	High salt content in the finalDNA eluate	Precipitate DNA with ethanol.
	Residual ethanol from the diluted wash solution	Centrifuge the column for 1 minute after the wash step to remove any residual wash solution.

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