NucleoGene Viral NA Extraction Kit

Instructions for Use

Release Date — 01.12.2019

CE IVD

REF

NG042 – 50 Test
100 Test
250 Test

For the isolation of DNA and RNA from human serum and plasma (EDTA) samples.
For nucleic acid isolation and purification.
Suitable for diagnostic use.

Kit Contents

	Material Supplied	50 Test	100 Test	250 Test
1.	Lysis&Binding Buffer†	17 ml	34 ml	85 ml
2.	Wash Buffer I *	17 ml	34 ml	85 ml
3.	Wash Buffer II *	11 ml	22 ml	55 ml
4.	Elution Buffer	5 ml	10ml	25ml
5.	Proteinase K	1 vial	1 vial	2 vial
6.	Carrier Mix	1 vial	1 vial	1 vial
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

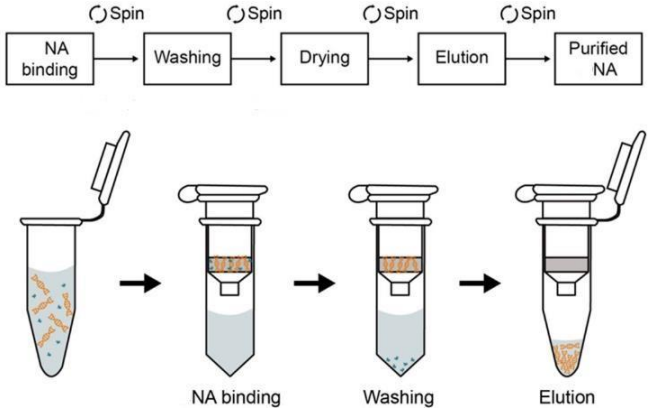
NucleoGene Viral NA Isolation Kit is specifically designed for the efficient purification of viral DNA and viral RNA from cell-free samples such as serum, plasma, body fluids and supernatant of viral infected cell cultures. The viral DNA / RNA is bound to the filter in the spin column and is released using a special buffer system. NucleoGene Viral NA Isolation Kit can be easily adapted to isolation devices and workstations using automatic spin columns. Thanks to the easy and fast (20 minute) working procedure of the kit, viral nucleic acids can be purified from several samples simultaneously with a simple laboratory infrastructure. Purified DNA / RNA is suitable for various procedures such as RT-PCR, qRT-PCR, qPCR, detection of viruses, viral load detection and viral genotyping.

Product Usage Limits

- NucleoGene Viral NA Isolation Kit is intended for purification of viral nucleic acids from samples such as human blood, plasma and serum. 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 viral nucleic acid from human body fluids 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 Viral NA Isolation Kit provides a fast, simple and cost-effective method for the isolation of viral DNA / RNA from cell-free samples such as serum, plasma, body fluids and supernatant of virus-infected cell cultures. 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 RNase-free elution buffer. The NucleoGene Viral NA Isolation Kit is suitable for a variety of routine applications including isolated viral DNA / RNA, Real-Time PCR / RT-PCR, DNA sequence analysis, PCR and other enzymatic reactions. The whole procedure can be completed in 20 minutes.



Purified DNA or RNA with complete isolation should be used immediately or a freezer at -20 to -80 ° 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

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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
- Isopropyl alcohol
- 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 & Binding 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.

Solution To make the wash buffers ready for use, add 96% or absolute ethanol to the amount indicated on the label. Then do not forget to shake for at least 10 seconds with your hand or vortex.

Protocol

Before starting NA 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.

Wash I: Add 11 mL of 96 % or absolute ethanol for 50 tests
Add 22 mL of 96 % or absolute ethanol for 100 tests
Add 55 mL of 96 % or absolute ethanol for 250 tests

Wash II: Add 44 mL of 96 % or absolute ethanol for 50 tests

Add 88 mL of 96 % or absolute ethanol for 100 tests

Add 220 mL of 96 % or absolute ethanol for 250 tests

Proteinase K: Add 1250 µl Nuclease Free Water for per vial

Carrier Mix:

*Add 200 µl Nuclease Free Water for vial (50 Test)

*Add 400 µl Nuclease Free Water for vial (100 Test)

*Add 1000 µl Nuclease Free Water for vial (250 Test)

1. Place the sample (200 µl serum, 200 µl plasma or 200 µl whole blood) into a 2 ml tube and vortex by adding 2 ml tube and vortex by adding 200 µl of Lysis&Bindig Buffer, 10 µl of Proteinase K and 4 µl of Carrier Mix, and then incubate for 10 minutes at 70 ° C.

2. After incubation, 100 µl of Lysis&Binding Buffer is added to the tube and with vortex is mixed for 30 seconds. Then incubate for 30 sec at room temperature.
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3. Mixture is transferred to the column. Centrifuge at 8,000 xg for 1 min. The liquid in the bottom tube is discarded and the column is placed in a collection tube.

4.500 µl of Wash Buffer I is added to the spin column. Centrifuge for 1 minute at 8,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a collection tube.

5.500 µl of Wash Buffer II is added to the spin column. Centrifuge for 1 minute at 8,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a collection tube.

6. Add 500 µl of Wash Buffer II to the spin column. Centrifuge for 1 minute at 8,000 x g. The bottom tube containing the liquid is discarded and the column is placed in collection tube.

OPTIONAL; If desired, this process can be performed two times.

7. The spin column is centrifuged at 16,000 xg for 30 sec to remove residual ethanol.

8. The spin column is transferred to a sterile 1.5 ml microcentrifuge tube. Add 50 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 8,000 xg for 1 minute. The received liquid contains NA and is ready to use. For long-term storage, the DNA is stored at -20°C, RNA -80 °C.

Swab Samples Protocol

Important; Pre-fill the needed amount of Elution Buffer in a 1.5 ml reaction tube and incubate the Elution Buffer at 70 °C until the elution step.

***Place the swab into a 1.5 ml reaction tube containing physiological saline (0,9 % NaCl) and incubate for 15 minutes at room temperature. Afterwards shake the swab vigorously, squeeze it and remove the swab. Proceed with 200 µl of the particle-free sample for further steps. Alternatively, cut the swab and transfer it to the tube and add 300 µl of Lysis & Binding Buffer, then continue the procedure.

1. Place the 200 µl sample into a 2 ml tube and vortex by adding 2 ml tube and vortex by adding 200 µl of Lysis&Bindig Buffer, 50 µl of Proteinase K and 4 µl of Carrier Mix, and then incubate for 10 minutes at 70 ° C.

Alternative; cut the swab and transfer it to the tube and add 300 µl of Lysis & Binding Buffer, 10 µl of Proteinase K and 4 µl of Carrier Mix, and then incubate for 10 minutes at 70 ° C. Then continue the procedure.

2. After incubation, 100 µl of Lysis&Binding Buffer is added to the tube and with vortex is mixed for 30 seconds. Then incubate for 30 sec at room temperature.

3. Mixture is transferred to the column. Centrifuge at 8,000 xg for 1 min. The liquid in the bottom tube is discarded and the column is placed in a collection tube.

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4. 500 µl of Wash Buffer I is added to the spin column. Centrifuge for 1 minute at 8,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a collection tube.

5. 500 µl of Wash Buffer II is added to the spin column. Centrifuge for 1 minute at 8,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a collection tube.

6. Add 500 µl of Wash Buffer II to the spin column. Centrifuge for 1 minute at 8,000 x g. The bottom tube containing the liquid is discarded and the column is placed in a collection tube.

OPTIONAL; If desired, this process can be performed two times.

8. The spin column is centrifuged at 16,000 xg for 30 sec to remove residual ethanol.

9. The spin column is transferred to a sterile 1.5 ml microcentrifuge tube. Add 50 µl of 70 °C pre warmed Elution Buffer to the center of the spin column and incubate for 1 minutes at room temperature.

10. The spin column is centrifuged at 8,000 xg for 1 minute. The received liquid contains NA and is ready to use. For long-term storage, the DNA is stored at -20°C, RNA -80 °C.

Troubleshooting Guide

Problem	Reason	Solution
Poor or Low DNA/RNA Recovery	RNase contamination	Provide an RNase-free working environment. For long-term storage freeze the DNA/RNA eluate at –20°C or –80°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 Carrier Mix	Confirm the concentration and storage temperature of the dissolved lyophilized RNA Carrier aliquots. Do not freeze-thaw one aliquot over 3-4 times.
Downstream applications using recovered DNA/RNA do not proceed	DNA/RNA concentration is too low	Precipitate DNA/RNA with alcohol, and then resuspend DNA/RNA in a smaller volume of Solution E or dH2O.
	High salt content in the final DNA/RNA eluate	Precipitate DNA/RNA 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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