NucleoGene
FFPE RNA Extraction Kit
Instructions For Use

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REF

Formalin-Fixed Paraffin Embedded Tissue Samples

For ribonucleic acid isolation and purification.
Not suitable for diagnostic use.
For professional use only.

NGE009 – 50 Test
100 Test
250 Test

Kit Contents

	Supplied Material	50 Test	100 Test	250 Test
1.	Proteinase K*	1 vial	2 vial	5 vial
2.	ProteinaseK Buffer	1 vial	2 vial	5 vial
3.	DNase Buffer	1 ml	2 ml	5 ml
4.	DNase I	1 vial	2 vial	5 vial
5.	Melt Buffer	25 ml	50 ml	125 ml
6.	Lysis Buffer†	10 ml	20 ml	50 ml
7.	Wash Buffer I*	18 ml	36 ml	90 ml
8.	Wash Buffer II*	12ml	24 ml	2x30 ml
9.	Binding Buffer†	20ml	40 ml	100 ml
10.	Elution Buffer	5 mL	10 ml	25 ml
11.	Spin Column	50	100	250
12.	Collection Tubes	50	100	250
13.	User's Guide	1	1	1

*Look at''Notes Before Starting" for preparation of Proteinase K and Wash Buffer.

†Precipitation may occur during storage. Heat to 50 °C dissolve the precipitate formed before use.

Storage Conditions & Robustness

Kit components should be stored at room temperature (15-25°C) excluding Proteinase K. Do not expose Kit to direct sunlight. Proteinase K should be stored at -20 °C. When used under the seconditions, the kit can be stored for 12 months without any performance loss.

Intended Use

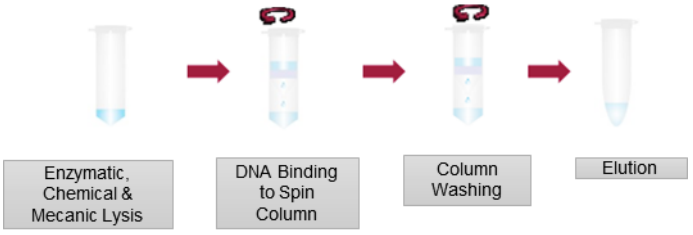
NucleoGene FFPE RNA Extraction Kit consists of silica-based membrane and special lysis buffer system for efficient RNA extraction from Formalin-Fixed Paraffin Embedded Tissue samples. This kit is specifically designed for the isolation and purification of RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Purified RNA is suitable for downstream applications such as reverse transcription, RT-PCR, and real-time quantitative RT-PCR (qRT-PCR). The NucleoGene FFPE RNA Extraction Kit is intended for research use only. It is not intended for the diagnosis and treatment of the disease.

Product Usage Limits

- NucleoGene FFPE RNA Extraction Kit is for purification of nucleic acids of FFPE RNA samples. It cannot be used for clinical purposes. Whether the kit is suitable for the user's own experimental design is up to the user's discretion.
- In particular, the ability of this kit to isolate RNA from FFPE samples has been confirmed.
- The collection, transport and storage of the sample to be used are at least important as purification andare sensitive stages that may affect the results.
- It is designed for professional use by educated personnel only.
- Kits or any kit components with different lot numbers should not be used together.

Introduction

The NucleoGene FFPE RNA Extraction Kit was developed to be a fast and convenient method for the isolation of total RNA from formalin-fixed paraffin-embedded (FFPE) tissue samples. The working principle of the kit is based on the ability of RNA to bind to glass fiber (silica) membranes in the presence of high concentrations of chaotropic salts. The kit has superior performance, FFPE sample tissue sections are first deparaffinized with the Melt Buffer method, then a short incubation with Lysis Buffer at a higher temperature, followed by incubation in a solution consisting of Lysis Buffer and Proteinase K, to completely obtain RNA from the sections. and free ribonucleic acids partially reverse the formalin cross-linking, increasing RNA yield and quality in downstream experiments. In order to provide suitable binding conditions for RNA in the kit, the lysate is mixed with Binding Buffer and ethanol, then the mixture is transferred to the spin column where the RNA is bound to the membrane and the impurities are removed with washing buffer. RNA is eluted with Elution Buffer.



The NucleoGene FFPE RNA Extraction Kit is optimized to use FFPE tissue in 3-8 sections with 10 µm thickness and 0.5-1 cm2 surface area (maximum 35 mg FFPE tissue can be used in total). The expected amount of RNA product will vary with the yield of the isolated sample, tissue type, size, and preservation methods. Approximately 1-2 micrograms of RNA are obtained per square centimeter of tissue section. Freshly purified RNA can be stored briefly at -20°C. -80 °C is recommended for long-term storage.

Warnings & Precautions

- All clinical specimens and residues and debris arising therefrom must be treated as potential infectious agents and disposed of accordingly.
- All samples should be prepared in Biosafety Level 1 and 2 or Class II Biosafety Cabins.
- All surfaces should be cleaned daily with a disposable paper towel or napkin by using freshly prepared Sodium Hypochlorite %20 Solution.
- Do not neglect to use laboratory safety consumable materials such as disposable gloves, goggles, visor, disposable cuffs, and disposable mask.
- If any of the kit components come into contact with your skin, wash it with plenty of water immediately. In case any of the kit components come into contact with your mucous membranes such as your eyes or mouth, wash the contact part with plenty of water again; and go to the doctor. If possible, prefer filtered pipette tips.
- Do not mix the kit solutions or waste appeared during isolation with bleach.
- Keep Kit out of contamination sources such as DNA and RNA, especially amplified nucleic acid.
- Do not mix solutions with different lot numbers or do not use or combine products from other companies.
- For more information, please apply the Material Safety Data Sheet(MSDS), which you can request from www.nucleogene.com

Other Required Materials

- Thermal Blockorwater bath
- Ethanol (%96-100)
- Xylene
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Mikrocentrifuge tubes (2.0 ml)
- Micropipette set and sterile filtered micropipette tips
- Spatula

Notes Before Starting

If the kit is kept in a non-air-conditioned environment, Lysis buffer solution can precipitate at room temperature in cold weather. In such a case to dissolve the precipitate, the Lysis buffer solution is held at 50°C in a water bath until dissolving completely. Be sure of all solutions are at the room temperature before and after protocol implementation, if they are not, allow them to reach room temperature.

-Add 12 mL of absolute or 96 % ethanol in Wash I Buffer bottle for 50 tests.
-Add 24 mL of absolute or 96 % ethanol in Wash I Buffer bottle for 100 tests.
-Add 60 mL of absolute or 96 % ethanol in Wash I Buffer bottle for 250 tests.

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

Then, do not forget shaking with your hand or with vortex at least 10 seconds.

Proteinase K is supplied as lyophilizing. Before starting to use the kit, all lyophilized Proteinase K should be mixed with with 1 ml of Proteinase K Buffer.

DNase I is supplied as lyophilizing. Before starting to use the kit, all lyophilized DNase I should be mixed with 0.5 ml of Nuclease Free Water.

Protocol

Control the reagents

Before starting to RNA isolation, control reagents that are free of leakage. To mix the solutions, shake the reagents slightly. If rea-gents precipitate (specially Lysis solution), they dissolve at 50 ° C by heating.

Deparaffinization

Deparaffinization with NucleoGene Melt Buffer

1. Using a microtome or scalpel, section 3-8 pieces with a thickness of 5 ~ 10 µm and a surface area of 0.5 ~ 1 cm2.

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

3. Add **NucleoGene Melt Buffer** to the sample:
• For sections ⩽50 microns, add 300µl of **NucleoGene Melt Buffer**.
• For sections >50 microns, add 500µl of **NucleoGene Melt Buffer**.

Vortex to mix and incubate for 5 minutes at 80 °C. 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 supernatant (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 RNA Isolation

***Before starting RNA extraction, thaw Lysis Buffer and Bindig Buffer completely by heating them at 50 °C.**

1. Add 160 µL of Lysis Buffer add 20 µL of Proteinase K solution to the remaining precipitates at the bottom of the tube and incubate (with 500 rpm shaking) for 30 min at 56 °C.

2. Increase the temperature of the thermomixer to 80 C and continue the incubation for another 30 minutes while shaking at 500 rpm.

3. After incubation, mix 20 µl DNase Buffer and 10 µl DNase I per sample in another tube and add 30 µl to the tube containing the lysate after incubation. Incubate for 15 minutes at 37 °C.

4. Add 340 µL of Binding Buffer and 750 µL of ethanol (Absolute) to the tube, mix by vortexing.

5. Transfer 650 µL of the lysate to the RNA Spin Column (placed in a 2 mL collection tube), close the lid, and centrifuge at 11.000 xg for 30 sec.

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

7. Repeat steps 5 and 6 until all liquid has passed through the spin column.

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

9. Add 600 µL of Wash Buffer I to the RNA Spin Column, centrifuge at 11.000 xg for 30 sec.

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

11. Add 600 µL of Wash Buffer II to the RNA Spin Column, centrifuge at 11.000 xg for 30 sec.

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

13. Add 600 µL of Wash Buffer II to the RNA Spin Column, centrifuge at 11.000 xg for 30 sec.

14. Discard the liquid in the collection tube and place the spin column back in the collection tube, centrifuge for 3 minute at 13.000 xg.

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

16. Keep the lid of the RNA spin column open and incubate at 56°C for 3 minutes.

17. Transfer 50 ~ 100 µL of Elution Buffer pre-warmed at 56 °C to the center of the filter of the RNA Spin Column and incubate for 2 minutes at room temperature. **Be careful not to touch the pipette tip to the filtered part of the column.**

18. After incubation, centrifuge the tubes for 1 minute at 11.000 xg.

***If a more efficient RNA recovery is desired, the elution step can be completed twice as 50 ul+50 ul.**

19. The liquid remaining in the tube contains RNA and is ready to use. For long-term storage, RNA is stored at -80 °C.

Troubleshooting

Issue	reason	solution
Clogged Column	Overuse sample	If more than 40 mg of sample is used, increase the Proteinase K, Solution volumes by the same rate. Pass the lysate through a column.
	Incompleted lysis	Be sure for mixing well of sample and lysis solution.
Low RNA amount	Wrong washing	Verify that the wash solution concentrates are diluted with the specified volumes of ethanol. To prevent evaporation, close the caps of the bottles thoroughly.
	Weak elution	Reduce Elution volume.
	Clogged column	Lookup
Enzymatic reactions do not run with isolated RNA.	Low RNA concentration	Precipitate DNA with ethanol and then dissolved with low volume of Elution.
	High salt content of isolated RNA	Precipitate RNA with ethanol.
	Ethanol remaining from washing buffer	After washing stage, centrifuge the spin column to remove remaining ethanol.