NucleoGene QuickEx Micro RNA Extraction Kit

Instructions for Use

Release Date— 10.12.2021

REF

For isolation and purification of RNA from Blood, Cell and Tissue samples.
For research use only.
Not suitable for diagnostic use.
For professional use only.

NGE036– 50 Test
100 Test
250 Test

Kit Contents

	Supplied Material	50 Test	100 Test	250 Test
1.	Lysis Buffer †	40 ml	80 ml	200 ml
2.	Wash Buffer I*	20 ml	40 ml	100 ml
3.	Wash Buffer II*	7 ml	14 ml	35 ml
4.	Enhancer	1 vial	1 vial	1 vial
5.	DNase I*	1 vial	1 vial	1 vial
6.	DNase I Buffer	2.25 ml	4.5 ml	11.25 ml
7.	Elution Buffer	5 ml	10 ml	25 ml
8.	Spin Column	50	100	250
9.	Collection Tubes	50	100	250
10.	User's Guide	1	1	1

*Look at''Notes Before Starting" for preparation DNase and Wash Buffer.

†Precipitation may occur during storage. Heat to dissolve the precipitate formed before use. Lysis Buffer should be stored at +4° C away from light.

Storage Conditions & Robustness

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

Intended Use

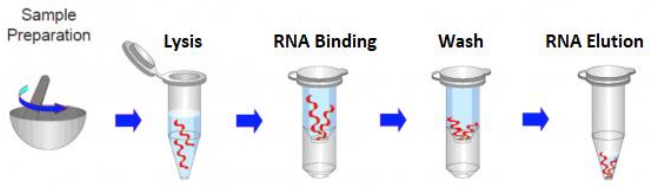
NucleoGene QuickEx Micro RNA Extraction Kit includes spin column and special lysis buffer system for effectively micro RNA(including mirna and small RNA) extraction in 8 minutes from blood(serum, plasma, whole blood), cell and tissue samples. Specifically designed for RNA isolation and purification. Suitable for downstream applications such as purified RNA, RT-PCR, northern blotting, primer extension, mRNA selection, cDNA synthesis. Real Time qualitative and quantitative RT-qPCR and others application. NucleoGene QuickEx Micro RNA Extraction Kit is designed for research use only. It is not for diagnosis and treatment of disease.

Product Usage Limits

- NucleoGene QuickEx Micro RNA Extraction Kit is for purification of RNA of blood, cell and tissue 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 of blood, cell and tissue 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

NucleoGene QuickEx Micro RNA Extraction Kit provides a quick and easy spin column system for purifying and enriching RNAs and other small cellular RNAs from a wide variety of blood, tissue and cells. Since RNAs are vital for regulating gene expression, this kit is optimized for isolation of RNA molecules while removing minimizing genomic DNA contamination for improved sensitive downstream applications. NucleoGene QuickEx Micro RNA Extraction Kit is improved for cell RNA extraction effectively from blood,cell and tissue samples to be a quick and useful method. Working principle of Kit depends on binding of RNA with its especially improved solutions to spin columns. The superior performance of the kit is due to its ability to lyse the cell walls of blood, cell and tissue samples, as well as its ability to remove PCR inhibitors present in the sample with solutions specially improved for , cell and tissue samples. While wash buffer optimized specifically is improved for purified nucleic acids especially from protein and other inhibitors, the Lysis Buffer solution is improved for lysing the cell wall and remove the inhibitors from the sample. With the complete the isolation process, RNA is obtained at high efficiency and purity.



Purified RNA with completed isolation should be used immediately or a freezer at -80 °C should be preferred 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

- Ethanol (%96-100)
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Microcentrifuge 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.

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DNase I is shipped at room temperature and should be stored at -20° C for extended periods after receiving the kit. Add absolute ethanol to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.Prepare Phosphate Buffered Saline (PBS, pH7.2) and 0.10-0.25% Trypsin for cultured cells.Yield and quality of RNA will be higher when fresh samples or samples which have been flash frozenand stored at -80° C are used.

Preparation of Solutions;

Wash Buffer I: Add 20 ml of 96 % or absolute ethanol for 50 tests
Add 40 ml of 96 % or absolute ethanol for 100 tests
Add 100 ml of 96 % or absolute ethanol per bottle for 250 tests

Wash Buffer II: Add 28 ml of 96 % or absolute ethanol for 50 tests
Add 56 ml of 96 % or absolute ethanol for 100 tests
Add 140 ml of 96 % or absolute ethanol per bottle for 250 tests

DNase I: Add 250 µl of Nuclease Free Water for 50 tests
Add 500 µl of Nuclease Free Water for 100 tests
Add 1250 µl of Nuclease Free Water for 250 tests

Enhancer: Add 200 µl of Nuclease Free Water for 50 tests
Add 400 µl of Nuclease Free Water for 100 tests
Add 1000 µl of Nuclease Free Water for 250 tests

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 reagents precipitate (specially Lysis solution), they dissolve at 50 ° C by heating.

Sample Preparation

Liquids Samples
Add an appropriate volume of Lysis Buffer to a liquid sample and mix thoroughly (see table below). To remove particulate debris (if any), centrifuge and transfer the supernatant into an RNase-free tube.

Sample	Sample Volume	Add Lysis Buffer
Biological liquid (blood, plasma, serum, WBCs, FACs, etc.) or Reaction clean-up (DNase I treated RNA, in vitro transcription, labeling, etc.).	100 µl	≥ 300 µl

Tissue(Tough-to-lyse samples)
Tough-to-lyse samples (see table below) can be homogenized in ≥ 800 µl Lysis Buffer with a mortar/pestle, syringe, tissue grinder, or bead beating with a high-speed homogenizer. To remove particulate debris from homogenized tissue, centrifuge and transfer the supernatant into a new nuclease-free tube.

Tissue	Gram(+) bacteria	Pathogen (microbes in tissue)
animal: high yield (≤ 25 mg) animal: low yield (≤ 50 mg) plant (≤ 100 mg)	bacteria (≤ $5x10^8$) yeast (≤ $5x10^7$)	animal/insect, plant (≤ 25 mg)

Cell

Lyse animal or gram(-) bacteria cells* directly in a culture dish or resuspend pelleted cells in an appropriate volume (see table below) of Lysis Buffer and mix thoroughly.**

*** For cell suspensions, add 3 volumes of Lysis Buffer to 1 volume of cell suspension.**
**** For direct lysis in a dish, add 100 µl for each cm^2 of culture surface area.**

Animal	Gram(-) bacteria	Add Lysis Buffer
≤ 10^5	-	≥ 100 µl
≤ 10^6	≤ 10^8	≥ 300 µl
≤ $5x10^6$	≤ $5x10^8$	≥ 600 µl

***Adherent Cultured Animal Cells**
• Remove the culture medium and wash cells in PBS.
• Aspirate PBS then add 0.10-0.25% Trypsin in PBS.
• Once cells have detached, add the medium and transfer to a 15 ml centrifuge tube.
• Proceed with Suspension Cultured Animal Cells.

***Suspension Cultured Animal Cells**
• Transfer cells (up to 1 x 10^6) to a 2 ml microcentrifuge tube.
• Harvest by centrifugation for 5 minutes at 300 x g.
• Carefully remove the supernatant completely by aspiration then proceed to Lysis.

***Bacterial Cells**
• Transfer 2 ml bacterial culture (up to 1 x 10^6) to a 2 ml microcentrifuge tube.
• Harvest by centrifugation for 5 minutes at 14000 x g.
• Carefully remove the supernatant completely by aspiration then proceed to Lysis.

***Yeast Cells**
• Transfer 2 ml yeast culture (up to 1 x 10^6) to a 2 ml microcentrifuge tube.
• Harvest by centrifugation for 5 minutes at 14000 x g.
• Carefully remove the supernatant completely by aspiration then proceed to Lysis.

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RNA EXTRACTION

***Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 1 min, unless specified.**

1. Ensure that the sample and Lysis Buffer and 4 ul Enhancer are fully mixed and 10 minutes incubation at room temperature, later centrifuge for 2 minutes at 14,000 xg as solid particles will clog the column. Carefully withdraw the supernatant and transfer it to a new (1.5-2 ml) microcentrifuge tube.

2. Add an equal volume ethanol (95-100%) (eg transferred supernatant 250 ul+250 ul ethanol) to a sample lysed in Lysis Buffer and mix (vortex 1 min or pipetting) thoroughly.

3. Transfer the mixture into a Spin Column in a Collection Tube and 11.000 xg 30 seconds centrifuge. Empty the collection tube and place the spin column back in the collection tube.

DNase I treatment (optional)
* In an RNase-free tube (not included), add 5 µl DNase I (6 U/l), 45 µl DNase I Buffer and mix by gentle inversion. Add the mix directly to the column matrix. Incubate at room temperature (20-30°C) for 15 minutes. Proceed to step 4.

4. Add 400 µl of Wash I Buffer to the Spin Column and centrifuge at 11,000 xg for 30 seconds.

5. Add 400 µl of Wash I Buffer to the Spin Column and centrifuge at 11,000 xg for 30 seconds. Empty the collection tube and place the spin column back in the collection tube.

6. Add 700 µl of Wash II Buffer to the Spin Column and centrifuge at 14,000 xg for 1 min. Discard the collection tube and place the spin column in a new nuclease-free microcentrifuge tube (1.5-2 ml).

7. To elute the RNA, add 50 µl of Elution Buffer directly to the center of the spin column and incubate 2 min, after the incubation centrifuge at 11,000 xg for 2 min.

***Alternatively, for highly concentrated RNA use ≥ 30 µl elution.**
The eluted RNA can be used immediately or stored frozen (-80 °C)

Troubleshooting

Precipitation, viscous lysate	**Incomplete lysis and/or high-mass input:** **- If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent.**
Low purity **(A260/A230 nm, A260/A280 nm)**	**Sample handling:** **- Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time.** **Incomplete lysis and/or cellular debris:** **Increase the volume of Lysis Buffer to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.**
Low yield	**Sample input:** **- Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised RNA recovery. Use less input material and/or increase Lysis Buffer.** **High-protein content (blood, plasma/serum, etc.)** **- Perform Proteinase K treatment to the sample prior to adding Lysis Buffer**
DNA contamination	**To remove DNA:** **- Perform in-column DNase I treatment or perform DNase I treatment post-purification then repurify the treatedsample.** **- For future preps, increase the volume of Lysis Buffer to ensure complete lysis and homogenization of the sample.**
RNA degradation	**To prevent RNA degradation:** **- Immediately collect and lyse fresh sample into Lyisis Buffer to ensure RNA stability. Homogenized samples in Lysisi Buffer can be stored frozen for later processing.**

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