NucleoGene
Total RNA Extraction Kit
Instructions for Use

Release Date— 01.12.2019

REF

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

NGE007– 50 Test
100 Test
250 Test

Kit Contents

	Supplied Material	50 Test	100 Test	250 Test
1.	Lysis Buffer †	35 ml	70 ml	175 ml
2.	Wash Buffer I	21 ml	42 ml	105 ml
3.	Wash Buffer II*	11 ml	22 ml	28x2 ml
4.	DNase I	1 vial	1 vial	1 vial
5.	DNase I Buffer	2.5 ml	5 ml	12.5 ml
6.	Elution Buffer	5 ml	10 ml	25 ml
7.	Spin Column	50	100	250
8.	Collection Tubes	50	100	250
9.	User's Guide	1	1	1

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

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

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

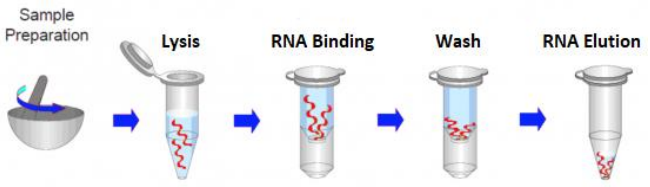
NucleoGene RNA Isolation Kit includes spin column and special lysis buffer system for effectively Total RNA extraction from , blood, cell and tissue samples. Specifically designed for Total RNA isolation and purification. Suitable for downstream applications such as purified Total RNA, RT-PCR, northern blotting, primer extension, mRNA selection, cDNA synthesis., RealTime qualitative and quantitative RT-qPCR and others application. NucleoGene Total RNAIsolation Kit is designed for research use only. It is not for diagnosis and treatment of disease.

Product Usage Limits

- NucleoGeneTotal RNA Isolation Kit is for purification of Total RNA of , 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 Total RNA of , cell and tissue sampleshas 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

NucleoGeneTotal RNA Isolation Kit provides a quick and easy spin column system for purifying and enriching RNAs(Total RNAs) and othersmall cellular RNAs from a wide variety of tissue and cells. Since Total RNAs are vital for regulating gene expression, this kit is optimized for isolation of Total RNA molecules while removingmini-mizing genomic DNA contamination for improved sensitive down-stream applications. NucleoGeneTotal RNA Isolation Kit is improved for cell RNA extraction effectively from ,cell and tissue samplesto be a quick and useful method. Working principle of Kit depends on binding of RNA with its especially improved solutions to spin col-umns. The superior performance of the kit is due to its ability to lyse the cell walls of , 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, Total 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.

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Other Required Materials

- Thermal Block or water bath
- 2-Mercaptoethanol
- Red Blood Cell Lysis Buffer
- Lyticase (0,5 mg/ml)
- Lysozyme (10mg/ml)
- Ethanol (%96-100)
- 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.

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 I: Add 14 ml of 96 % or absolute ethanol for 50 tests
Add 28 ml of 96 % or absolute ethanol for 100 tests
Add 70 ml of 96 % or absolute ethanol per bottle 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 110 ml of 96 % or absolute ethanol per bottle for 250 tests

DNase I: Add 275 µl of Nuclease Free Water for 50 tests
Add 550 µl of Nuclease Free Water for 100 tests
Add 1375 µl of Nuclease Free Water for 250 tests

Protocol

Control the Reagents

Before starting to Total 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

Whole Blood

•Add 1 ml Red Blood Cell Lysis Buffer to a sterile 1.5 ml reaction tube.
•Add 500 µl human whole blood and mix by inversion. Do not vortex.
•Place the tube on a rocking platform or gyratory shaker for 10 min at +15 to +25° C. Alternatively, manually invert the sample periodically for 10 min.
•Centrifuge for 5 min at 500 × g in a standard table top centrifuge.
•With a pipette, carefully remove and properly dispose of the clear, red supernatant. Add 1 ml Red Blood Cell Lysis Buffer to the white pellet and mix by “flicking” the tube until the pellet is resuspended. Do not vortex.
•Centrifuge for 3 min at 500 × g. Carefully remove and properly dispose of the supernatant, particularly the red ring of blood cell debris that forms around the outer surface of the white pellet.

After these procedures, continue with the Culture Cell Total RNA Isolation protocol.

Tissue
Remove frozen or fresh tissue samples from storage.
• Transfer up to 50 mg of fresh or frozen animal tissue to a 1.5 ml microcentrifuge tube then tissue should be powder in liquid nitrogen.
•50-100 mg of plant tissue should be used and the tissue should be powder in liquid nitrogen.
NOTE: Ensure frozen tissue does not thaw prior to adding Lysis Buffer.

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 10^7) 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 10^7) 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 10^7) 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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Plant and Mammalian Tissue Total RNA Isolation

1.Transfer (100 mg powder plant or up to 50 mg animal) of tissue sample to a 2 ml tube, add 600 µl of Lysis Buffer then 30 s vortex.

2.Add 6 µl of 2-mercaptoethanol to the tube and pipetting for 5 times then incubate for 10 minutes at room temperature.

3.After incubation, centrifuge the tube at 13.000xg for 3 minutes. Then transfer the supernatant to a new 2 ml of the tube, add equal volume absolute ethanol and mix (pipetting).

4.Subsequently, transfer all liquid to spin column placed into the collection tube and centrifuge spin columns at 13.500xg for 30 sec.

5.Place the spin columns to a collection tube, add 700 µl Wash Buffer I and centrifuge at 13.500xg for 30 sec.

6.Place the spin columns to a collection tube, add 500 µl Wash Buffer II and centrifuge at 13500xg for 30 sec. (this process is repeated 2 times).

7.Place the spin columns to a collection tube and centrifuge at 14.000xg for for 2 minutes to remove Ethanol residue.

8.Place the spin columns into 1.5 ml microcentrifuge tubes. Transfer 50µl of preheated to 80 ° C Elution Buffer to spin column and incubate for 1 minute at room temperature.

9.After incubation, centrifuge spin columns at 13.500 x g for 1 minute. Throw the spin columns and close the cap of tubes. Liquid in the tube contains RNA and ready for use. RNA is kept at -80 ° C for long time storage.

Optional Step: DNA Digestion in Solution

1.Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water (Elutution Buffer)	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2.Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.

3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) theincubate the microcentrifuge tube at 65°C for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis,repurify the RNA sample by using the NucleoGeneBig Dye Cleanup Kitinstead of stopping the reaction with EGTA.

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Culture or Blood Cell Total RNA Isolation

1.2 ml tube containing culture cells (pellet fraction), add 250 µl of Lysis Buffer then resuspend cells by pipette until the pellet is dissolved completely.

2.Add 2.5 µl of 2-mercaptoethanol to the tube and pipetting for 5 times then incubate for 10 minutes at room temperature.

3.After incubation, centrifuge the tube at 14000xg for 1 minutes. Then transfer the supernatant to a new 2 ml of the tube, add equal volume absolute ethanol and mix (pipetting).

4.Subsequently, transfer all liquid to spin column placed into the collection tube and centrifuge spin columns at 14000xg for 1 minutes.

5.Place the spin columns to a collection tube, add 700 µl Wash Buffer I and centrifuge at 14000 x g for 30 second.

6.Place the spin columns to a collection tube, add 500 µl Wash Buffer II and centrifuge at 14000 x g for 30 second (this process is repeated 2 times).

7.Place the spin columns to a collection tube and centrifuge at maximum rate for 1 minutes to remove Ethanol residue.

8.Place the spin columns into 1.5 ml microcentrifuge tubes. Transfer 50µl of Elution Buffer to spin column and incubate for 1 minute at room temperature.

9.After incubation, centrifuge spin columns at 14000 x g for 2 minutes. Throw the spin columns and close the cap of tubes.Liquid in the tube contains RNA and ready for use. RNA is kept at -80 ° C for long time storage.

Optional Step: DNA Digestion in Solution

1.Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2.Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.

3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) the incubate the microcentrifuge tube at 65°C for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the NucleoGene Big Dye Cleanup Kit instead of stopping the reaction with EGTA.

Bacterial CellsTotal RNA Isolation

1.2 ml tube containing bacterial cells (pellet fraction), add 250 µl of Lysis Buffer then resuspend cells by pipetteuntil the pellet is dissolved completely.

2.Add 2.5 µl of 2-mercaptoethanol and 20 µl Lysozyme to the tube and pipetting for 5 times then incubate for 10 minutes at 55° C.

3.After incubation, centrifuge the tube at 14000xg for 1 minutes. Then transfer the supernatant to a new 2 ml of the tube, add equal volume absolute ethanol and mix (pipetting).

4.Subsequently, transfer all liquid to spin column placed into the collection tube and centrifuge spin columns at 14000xg for 1 minutes.

5.Place the spin columns to a collection tube, add 700 µl Wash Buffer I and centrifuge at 14000 x g for 30 second.

6.Place the spin columns to a collection tube, add 500 µl Wash Buffer II and centrifuge at 14000 x g for 30 second (this process is repeated 2 times).

7.Place the spin columns to a collection tube and centrifuge at maximum rate for 1 minutes to remove Ethanol residue.

8.Place the spin columns into 1.5 ml microcentrifuge tubes. Transfer 50µl of Elution Buffer pre-heated to to spin column and incubate for 1 minute at room temperature.

9.After incubation, centrifuge spin columns at 14000 x g for 2 minutes. Throw the spin columns and close the cap of tubes.Liquid in the tube contains RNA and ready for use. RNA is kept at -80 ° C for long time storage.

Optional Step: DNA Digestion in Solution

1.Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2.Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.

3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) the incubate the microcentrifuge tube at 65°C for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the NucleoGene Big Dye Cleanup Kit instead of stopping the reaction with EGTA.

Yeast Cells Total RNA Isolation

1.2 ml tube containing yeast cells (pellet fraction), add 250 µl of Lysis Buffer then resuspend cells by pipette until the pellet is dissolved completely.

2.Add 2.5 µl of 2-mercaptoethanol and 40 µl Lyticase to the tube and vortex for 30 seconds. Then incubate for 30 minutes at 37 °C.

3.After incubation, centrifuge the tube at 14000xg for 1minutes. Then transfer the supernatant to a new 2 ml of the tube, add equal volume absolute ethanol and mix (pipetting).

4.Subsequently, transfer all liquid to spin column placed into the collection tube and centrifuge spin columns at 14000xg for 1 minutes.

5.Place the spin columns to a collection tube, add 700 µl Wash Buffer I and centrifuge at 14000 x g for 30 second.

6.Place the spin columns to a collection tube, add 500 µl Wash Buffer II and centrifuge at 14000 x g for 30 second (this process is repeated 2 times).

7.Place the spin columns to a collection tube and centrifuge at maximum rate for 1 minutes to remove Ethanol residue.

8.Place the spin columns into 1.5 ml microcentrifuge tubes. Transfer 50 µl of Elution Buffer to spin column and incubate for 1 minute at room temperature.

9. After incubation, centrifuge spin columns at 14000 x g for 2 minutes. Throw the spin columns and close the cap of tubes. Liquid in the tube contains RNA and ready for use. RNA is kept at -80 °C for long time storage.

Optional Step: DNA Digestion in Solution

3.Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

4.Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.

3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) the incubate the microcentrifuge tube at 65°C for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the NucleoGene Big Dye Cleanup Kit instead of stopping the reaction with EGTA.

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Troubleshooting

Clogged Column	• Insufficient disruption and/or homogenization/too much starting material • Centrifugation temperature was too low (should be 20ºC to 25ºC)
Low RNA Yield	• RNA still bound to the Spin Column membrane • Ethanol carryover
RNA Degradation	• Harvested sample not immediately stabilized/inappropriate handling of starting material • RNase contamination

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