NucleoGene DNA&RNA&Protein Extraction Kit

Instructions For Use

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REF

For isolation and purification o f Deoxyribonucleic acid, Ribonucleic acid and Protein from cell, tissue and blood samples.
For research implementation only.
Not suitable for diagnostic use.
For professional use only.

NGE011– 50 Test
100 Test
250 Test

Kit Contents

	SuppliedMaterial	50 Tests	100 Tests	250 Tests
1.	Proteinase K*	1 vial	2 vial	5 vial
2.	Lysis Buffer†	18 ml	36 ml	90 ml
3.	Wash Buffer I	16 ml	32 ml	80 ml
4.	Wash Buffer II	12 ml	24 ml	30 ml x 2
5.	Protein Precipitation Buffer	40 ml	80 ml	200 ml
6.	DNase I	1 vial	2 vial	5 vial
7.	DNase I Reaction Buffer	1 vial	1 vial	1 vial
8.	ElutionBuffer	20 ml	40 ml	100 ml
9.	DNA Spin Column (White)	50	100	250
10	RNA Spin Column (Green)	50	100	250
11	Collection Tubes	100	200	500
12	User's Guide	1	1	1

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

†Precipitation may ocur during storage. Heat to dissolve the precipitate for 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 conditions, the kit can be stored for 12 months without any performance loss.

Intended Use

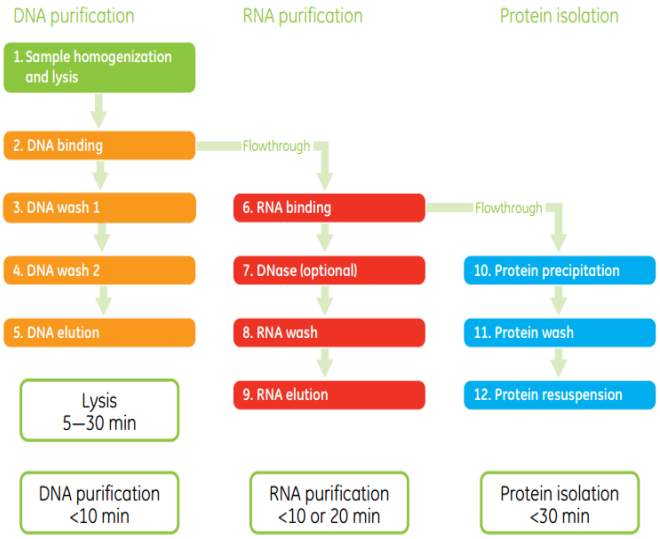
NucleoGene DNA&RNA&Protein Extraction Kitis designed for the rapid extraction of genomic DNA, total RNA and total denatured protein from a single undivided sample starting from as little as 1 mg of animal tissue, 0.3 million cultured mammalian cells or 200 ul blood samples. The protocols for extraction from both tissues and cell lines utilizethe same buffers. Although the protocols are rapid, they have beendesigned to minimize shearing of genomic DNA, maintain the integrityof the RNA and maximize proteome representation.The entire procedure can be completed in as little as 45 minutes toyield genomic DNA, total RNA and denatured total protein which arecompatible with most molecular biology applications.

Product Usage Limits

- NucleoGene DNA&RNA&Protein Extraction Kitis for purification of nucleic acids of cell, tissue and blood 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 DNA&RNA&Protein from cell, tissue and blood 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

NucleoGeneDNA&RNA&Protein Extraction Kitis improved for DNA&RNA&Protein extraction effectively from cell, tissue and blood samples to be a quick and useful method. Working principle of Kit depends on binding of DNA&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 further-processed cell, tissue and blood samples, as well as its ability to remove PCR inhibitors present in the sample with solutions specially improved for cell, tissue and blood samples. While wash buffer optimized specifically is improved for purified nucleic acids especially from 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, DNA&RNA&Protein is obtained at high efficiency and purity.



NucleoGeneDNA&RNA&Protein Extraction Kits optimized for use in cell, tissue and blood samples. Purified DNA and RNA with completed isolation should be used immediately or a freezer at -20 ° C and -80 ° C should be preferred for long-term storage.

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Warnings&Precautions

- All clinical specimens and residues and debris arising there from 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.
- Allsurfacesshould be cleaneddailywith 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 Block or water bath
- Ethanol (%96-100)
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Mikrocentrifuge tubes (2.0 ml)
- Micropipette set and sterile filtered micropipette tips
- Spatula
- Nuclease-free 20-gauge needle and syringe (1 ml) if usingmortar & pestle or for cell lysate homogenization.
- 2-Mercaptoethanol (14.3 M or >98%)
- Red Blood Cell Lysis Solution
- Absolute Ethanol (molecular biology grade)
- Nuclease –free Water (molecular biology grade)
- Absolute Acetone (molecular biology grade)

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.

Lysis buffer; 20 ml (Remove aliquot and add 2-Mercaptoethanol to aliquot before use). For each sample being processed, aliquot 350 µl of Lysis Buffer to a fresh DNase&RNase-free tube and add 3.5 µl of 2-Mercaptoethanol. Prepare a fresh aliquot, based on total numberof samples being processed, each time the kit is used. 2-Mercaptoethanol is not stable in Lysis buffer.

Do NOT add 2-Mercaptoethanol directly to the kit bottle of Lysis buffer. Lysis bufferwithout 2-Mercaptoethanol is required during the protocol.

DNase I; is supplied as lyophilizing. Each lyophilized DNase I tube should be mixed with 0,5 ml of Nuclease Free Water.

Wash Buffer I;
Add 6 ml Ethanol before 1st time use for 50 Test
Add 12 ml Ethanol before 1st time use for 100 Test
Add 30 ml Ethanol before 1st time use for 250 Test

Wash Buffer II;
Add 48 ml Ethanol before 1st time use for 50 Test
Add 96 ml Ethanol before 1st time use for 100 Test
Add 120 ml Ethanol before 1st time use each bottle for 250 Test

Proteinase K is supplied as lyophilised. Before starting to use the kit, all lyophilized Proteinase K should be mixed with Proteinase K buffer (Each lyophilized Proteinase K tube should be mixed with 1 ml of Proteinase K Buffer).

Protocol

Control the reagents

Before starting to DNA&RNA&Proteinextraction, 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.

DNA&RNA&Protein Extraction

Sample Homogenization & Lysis

1.Tissue homogenization &Lysis using a rotor-stator homogenizer
a. Select proper sized generator and suitablesized tube for homogenization of a small volume of sample (i.e., 350 µl or more)
b. Add 350 µl of Lysis buffer containing 2-Mercaptoethanol to the tube.
c. Add tissue sample (1 – 20 mg).
d. Homogenize the tissue according to instruments user manual. Visually inspect the prepared homogenate and ensure thorough homogenization (No tissue pieces should be visible).
e. Proceed immediately to Step 2 or store at-80° C for up to 6 months.
f. Add 20 µl of Proteinase K and incubate at 60° C for 30 minutes.

Tissue homogenization & Lysis using amortar & pestle and a needle & syringe

a. Dispense sufficient liquid nitrogen to coverthe bottom of a mortar.Immediately placetissue (1 – 20 mg) in liquid nitrogen, and grindthe sample to a fine powder in the presenceof liquid nitrogen.
b. Allow the liquid nitrogen to evaporate but do not allow the tissue to thaw.
c. Add 350 µl of Lysis buffer containing 2-Mercaptoethanol and grind further withthe pestle until no visible tissue pieces are present.
d. Pass lysate at least 5 times through a DNase&RNase-free 20-gauge needle fitted with1 ml sterile syringe.
e. Proceed immediately to Step 2 or store at-80° C for up to 6 months.
f. Add 20 µl of Proteinase K and incubate at 60° C for 30 minutes.

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Cell homogenization & Lysis

a. Pellet up to 5 × 10^6 cultured cells in a 1.5 mlmicrocentrifuge tube by centrifugation for1 minute at 11 000 × g.
b. Completely remove the supernatant by aspiration.
c. Wash the pellet with 500 µl PBS
d. Spin for 1 minute at 11 000 × g.
e. Completely remove the supernatant by aspiration.
Cell pellet can be stored at -80° C for up to 12months.
f. Add 350 µl of Lysis buffer containing 2-Mercaptoethanol.
g. Pipet up and down to resuspend the cell pellet.
h. Homogenize by passing lysate at least 5 times through a DNase&RNase-free 20-gauge needle fitted with 1 ml sterile syringe.
f. Proceed immediately to Step 2 or store at-80° C for up to 6 months.
h. Add 20 µl of Proteinase K and incubate at 60° C for 30 minutes.

Whole Blood & Lysis

a. Transfer 200 ul of blood stored in an EDTA tube into a 1.5 ml tube. Add 1000 ul volumes of ice-cold Red Blood Cell Lysis Solution and mix thoroughly by vortexing. For other mammalian cell types pellet the cells.
-Incubate at room temperature for 5 to 10 mins and vortex frequently (3 to 5 times) during incubation.
-Spin at 250 X G for 3 mins and discard supernatant without disturbing the pellet.
-Add 400 ul of ice-cold Red Blood Cell lysis solution to the pellet and mix thoroughly by vortexing
-Spin at 250 X G for 3 mins and discard supernatant without disturbing the pellet. Add 20 ul of Proteinase K solution.
-Pipet up and down to resuspend.
c. Add 350 µl of Lysis buffer containing 2-Mercaptoethanol and incubate at 60° C for 10 minutes.
d. Proceed immediately to Step 2 or store at -80° C for up to 6 months.

IMPORTANT
If you are unable to perform all these homogenization processes, add 20 microliters of Proteinase K to the lysis buffer in the specified amounts and incubate at 60°C for 30-60 minutes. Then take the supernatant by centrifuging at 12000xg for 1 minute and proceed with the DNA isolation protocol.

Genomic DNA isolation

2. DNA binding
a. Place a new spin column into a 2 ml Collection tube (provided).
b. Transfer the homogenized lysate from Step 1(350 µl) to the column.
c. Spin for 1 minute at 11 000 × g.
d. Save the flow through for RNA and Protein isolation at room temperature. (Refer to Step 6 or Step 10 for further processing)
e. Transfer the column to a new 2 ml Collection tube (provided). Proceed to Step 3.

3. DNA Wash I
a. Add 500 µl of Wash I Buffer to the column.
b. Spin for 1 minute at 11 000 × g. Discard the flow through.
c. Place the column back into the sameCollection tube.

4. DNA Wash II
a. Add 500 µl of Wash II Buffer to the column.
b. Spin for 1 minute at 11 000 × g. Discard the Collection tube and its contents.

5. DNA Elution
a. Add 100 µl of Elution Buffer to the center of the column.
b. Spin for 1 minute at 11 000 × g to collect the purified DNA as the flow through in the 1.5 ml microcentrifuge tube.
c. Discard the column and store purified DNA at -20° C or -80° C. If not subjected to repeated freeze-thaw cycles, DNA is stable for 12 months.
d. Proceed immediately to Step 6 to isolate RNA.

Total RNA isolation

6. RNA Binding

a. Place a new spin column in a new Collection tube
b. Add 350 µl of Absolute Acetone or Ethanol to the flowthrough from Step 2d. Mix well by pipetting up and down several times. Transfer the entire mixture to the mini column .
c. Spin for 1 minute at 11 000 × g.
d. Save the flowthrough for protein isolation at room temperature. (Refer to Step 10 forfurther processing)
e. Transfer the column to a new Collection tube(provided). Proceed to Step 7 or Step 8 as required.

7.DNase treatment (optional)

Crude lysate containing more than 20 µg DNA (forexample, when 10 mg rat liver or 3 million HeLaCells are processed) may require use of DNase to remove residual genomic DNA in the isolated RNA.

a. In a RNase-free microcentrifuge tube, add 10 µl of reconstituted DNase I, 20 µl of DNase Reaction Buffer and 70 µl RNase-free water. Mix by flicking the tube. Do not vortex.
b. Apply 100 µl of diluted DNase directly on the center of membrane of the column. Incubatefor 10 minutes at room temperature. Proceed to Step 8.

8. RNA Wash

a. Add 500 µl of Wash Buffer II to the column.
b. Spin for 1 minute at 11 000 × g. Discard the Collection tube and its contents.Please check that no residual flowthrough remains in the column outlet. If any remains, empty the Collection tube, put the column back, repeat Step 8b.
c. Transfer the column to a fresh RNase-free1.5 ml microcentrifuge tube (user supplied).

9. RNA Elution

a. Add 100 µl Elution Buffer to thecenter of the column.50 µl or less of Elution Buffer can be used for achieving more concentrated RNA elution.
b. Spin for 1 minute at 11 000 × g to collect thepurified total RNA as the flowthrough in the Collection tube.
c. Discard the column and store purified RNAat -80° C. Purified RNA is stable for up to 12months. Proceed to Step 10.

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Total Protein Isolation

10. Protein Precipitation

a. Transfer the entire flow through(approximately 600 µl) from Step 6d to a new 1.5 ml microcentrifuge tube.Flowthrough containing total protein can bestored at -80° C for up to 6 months.
b. Add 600 µl of Protein precipitation Buffer.
c. Mix vigorously and incubate for 5 minutes atroom temperature to precipitate the proteins.
d. Spin for 10 minutes at full speed (minimum16 000 × g).
e. Carefully remove as much supernatant as possible by pipetting or decanting.

11.Protein Wash

a. Add 1 ml of distilled water to protein pellet(user supplied).
b. Actively disperse protein pellet by pipetting up and down at least 5 times.
c. Spin for 1 minute at full speed (minimum16 000 × g).

12. Protein Resuspension

a. Add 50–500 µl of 7M UreaBuffer
b. Incubate for 5 minutes at room temperature.

Note: 7 M Urea can be used for protein resuspension and further diluted to less than 3 M to be compatible with BCA or Bradfordassays.

Troubleshooting Guide

Problem	Possible cause	Suggestions
Low Genomic DNA Yield	Homogenization of tissue in complete	• Use of rotor-stator homogenizer is recommended. • For difficult to lyse tissues such as mouse or rat tails, crushing the animal tissue inliquid nitrogen is recommended prior to proceeding with DNA extraction.
	Column clogged due to overloading	Do not use more than 20 mg tissue as starting material for each sample. If necessary, split the tissue between two preparations.
Poor Quality of Isolated Genomic DNA	Too much tissue or too many cells used per sample	Ensure correct amounts used.
RNA isdegraded/no RNA obtained	RNase contamination	Create an RNase-free working environment. Wear gloves during all steps of the procedure, and change gloves frequently. Use of RNase-free,disposable polypropylene tubes is imperative. Keep tubes closed when ever possible during the preparation.Glassware should be oven baked for at least 2 hours at 250° C before use.
Poor RNA quality or yield	Reagents not prepared,stored or applied properly	• Reagents not properly stored. • Add the indicated volume of nuclease free water to DNase I vial and mix. Addthe indicated volume of Absolute Ethanolto Wash Buffer and mix • Reconstitute and store lyophilized DNase according to instructions given insection 5.1. • Sample and reagents have not been mixed completely. Vortex vigorously after each reagent has been added (except for DNase I). • Absolute Acetone was not added after lysis. Binding of RNA to the silica membrane is only effective in the presence of Absolute Acetone.
Clogged during RNA binding	Sample material in completely homogenized	• Centrifuge crude lysate at 11 000 × g to pellet any insoluble material that may clog the column. • Reduce the sample amount, increasethe time for the centrifugation steps,and/or increase the volume of Lysis buffer (Note: only load 350 µl of crude lysate onto the 1st spin column) • If clogging still occurs, take theremaining lysate off the mini column,discard it, and proceed with the wash step with Wash buffer.
Protein pelletcan not be resuspended	Incomplete salt removal during pellet wash step	Follow the protocol, break the proteinpellet with pipet tip during water wash for effective removal of salts.

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