Nucleogene Tri Reagent
Instructions for Use

Release Date— 01.12.2019

Store at RT

REF

For research use only.
Not suitable for diagnostic use.
For professional use only.

NGE023 50 ml
100 ml
200 ml

Description

Nucleogene Tri Reagent is a ready-to-use reagent, designed to isolate high quality total RNA (as well as DNA and proteins) from cell, blood and tissue samples of human, animal, plant, yeast, or bacterial origin, within one hour. Nucleogene Tri Reagent is a monophasic solution of phenol, guanidine, and other proprietary components which facilitate the isolation of a variety of RNA species of large or small molecular size. Nucleogene Tri Reagent maintains the integrity of the RNA due to highly effect' ve inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization. Nucleogene Tri Reagent allows for simultaneous processing of a large number of samples, and is an improvement to the single-step RNA isolation method.

Required materials not supplied

- Centrifuge and rotor capable of reaching 12,000 × g and 4°C
- Tubes Polypropylene microcentrifuge tubes
- Chloroform

Materials required for RNA isolation

- Water bath or heat block at 55–60°C
- Isopropanol
- Ethanol, 75%
- RNase-free water of 0.5% SDS
- (Optional) RNase-free glycogen

Materials required for DNA isolation

- Ethanol, 100%
- Ethanol, 75%
- 0.1 M sodium citrate in 10% ethanol
- 8 mM NaOH
- HEPES

Materials required for protein isolation

- (Optional) Dialysis membranes
- Isopropanol
- Ethanol, 100%
- 0.3 M Guanidine hydrochloride in 95% ethanol
- 1% SDS

Note; Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at –80°C or in liquid nitrogen until RNA isolation.

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Sample type	Starting material per 1 mL of Nucleogene Tri Reagent
Tissues	50–100 mg of tissue
Cells grown in monolayer	$1 \times 10^{5-1} \times 10^{7}$ cells grown in monolayer in a 3.5–cm culture dish (10 cm2)
Cells grown in suspension	$5–10 \times 10^{6}$ cells from animal, plant, or yeasty origin or 1×10^{7} cells of bacterial origin

Lyse Samples and Separate Phases

1. Lyse and homogenize samples in Nucleogene Tri Reagent according to your starting material.

• Tissues:
Add 1 mL of Nucleogene Tri Reagent per 50–100 mg of tissue to the sample and homogenize using a homogenizer.

• Cell grown in monolayer:
a. Remove growth media.
b. Add 0.3–0.4 mL of Nucleogene Tri Reagent per 1×10^{5}—10^{7} cells directly to the culture dish to lyse the cells.
c. Pipet the lysate up and down several times to homogenize.

• Cells grown in suspension:
a. Pellet the cells by centrifugation and discard the supernatant.
b. Add 0.75 mL of Nucleogene Tri Reagent per 0.25 mL of sample ($5–10 \times 10^{6}$ cells from animal, plant, or yeasty origin or 1×10^{7} cells of bacterial origin) to the pellet.

Note: Do not wash cells before addition of Nucleogene Tri Reagent to avoid mRNA degradation.

c. Pipet the lysate up and down several times to homogenize.

Note: The sample volume should not exceed 10% of the volume of Nucleogene Tri Reagent used for lysis.

STOPPING POINT Samples can be stored at 4°C overnight or at –20°C for up to a year.

2. (Optional) If samples have a high fat content, centrifuge the lysate for 5 minutes at 12,000 × g at 4–10°C, then transfer the clear supernatant to a new tube.

3. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.

4. Add 0.2 mL of chloroform per 1 mL of Nucleogene Tri Reagent used for lysis, then securely cap the tube.

5. Incubate for 2–3 minutes.

6. Centrifuge the sample for 15 minutes at 12,000 × g at 4°C. The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.

7. Transfer the aqueous phase containing the RNA to a new tube.

8. Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipette the solution out.

Save the interphase and organic phase if you want to isolate DNA or protein. See "Isolate DNA" or "Isolate proteins" on detailed procedures. The organic phase can be stored at 4°C overnight.

RNA Isolation

Precipitate the RNA

a. (Optional) If the starting sample is small (<10^6 cells or <10 mg of tissue), add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase.

Note: The glycogen is co-precipitated with the RNA, but does not interfere with subsequent applications.

b. Add 0.5 mL of isopropanol to the aqueous phase, per 1 mL of Nucleogene Tri Reagent used for lysis.

c. Incubate for 10 minutes.

d. Centrifuge for 10 minutes at 12,000 × g at 4°C. Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.

e. Discard the supernatant with a micropipettor.

RNA Washing

a. Resuspend the pellet in 1 mL of 75% ethanol per 1 mL of Nucleogene Tri Reagent used for lysis.

Note: The RNA can be stored in 75% ethanol for at least 1 year at –20°C, or at least 1 week at 4°C.

b. Vortex the sample br' efl¢ĵ then centrifuge for 5 minutes at 7500 × g at 4°C.

c. Discard the supernatant with a micropipettor.'

d. Vacuum or air dry the RNA pellet for 5–10 minutes.

Solubilize the RNA

a. Resuspend the pellet in 20–50 µL of RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution by pipetting up and down.

IMPORTANT! Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.

b. Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.

Proceed to downstream applications, or store the RNA at –70°C.

DNA Isolation

DNA Precipitation

a. Remove any remaining aqueous phase overlying the interphase. This is critical for the quality of the isolated DNA.

b. Add 0.3 mL of 100% ethanol per 1 mL of Nucleogene Tri Reagent used for lysis.

c. Cap the tube, mix by inverting the tube several times.

d. Incubate for 2–3 minutes.

e. Centrifuge for 5 minutes at 2000 × g at 4°C to pellet the DNA.

f. Transfer the phenol-ethanol supernatant to a new tube.

DNA Washing

a. Resuspend the pellet in 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 1 mL of Nucleogene Tri Reagent used for lysis.

b. Incubate for 30 minutes, mixing occasionally by gentle inversion.
Note: The DNA can be stored in sodium citrate/ethanol for at least 2 hours.

c. Centrifuge for 5 minutes at 2000 × g at 4°C.

d. Discard the supernatant with a micropipettor.

e. Repeat step 2a–step 2d once.

Note: Repeat step 2a–step 2d twice for large DNA pellets (>200 µg).

f. Resuspend the pellet in 1.5 – 2 mL of 75% ethanol per 1 mL of Nucleogene Tri Reagent used for lysis.

g. Incubate for 10–20 minutes, mixing occasionally by gentle inversion.

Note: The DNA can be stored in 75% ethanol at several months at 4°C.

h. Centrifuge for 5 minutes at 2000 × g at 4°C.

i. Discard the supernatant with a micropipettor.

j. Vacuum or air dry the DNA pellet for 5–10 minutes.

DNA Solubilization

a. Resuspend the pellet in 0.3–0.6 mL of 8 mM NaOH by pipetting• up and down.
Note: We recommend resuspending the DNA is a mild base because isolated DNA does not resuspend well in water or Tris buffer.

b. Centrifuge for 10 minutes at 12,000 × g at 4°C to remove insoluble materials.

c. Transfer the supernatant to a new tube, then adjust pH as needed with HEPES.

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Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C, adjust the pH to 7–8 with HEPES and add 1 mM EDTA.

Protein Isolation

Precipitate the proteins

a. Add 1.5 mL of isopropanol to the phenol-ethanol supernatant per 1 mL of Nucleogene Tri Reagent used for lysis.

b. Incubate for 10 minutes.

c. Centrifuge for 10 minutes at 12,000 × g at 4°C to pellet the proteins.

d. Discard the supernatant with a micropipettor.

Wash the proteins

a. Prepare a wash solution consisting of 0.3 M guanidine hydrochloride in 95% ethanol.

b. Resuspend the pellet in 2 mL of wash solution per 1 mL of Nucleogene Tri Reagent used for lysis.

c. Incubate for 20 minutes.
Note: The proteins can be stored in wash solution for at least 1 month at 4° C or for at least 1 year at –20°C.

d. Centrifuge for 5 minutes at 7500 × g at 4°C.

e. Discard the supernatant with a micropipettor.

f. Repeat step 2b–step 2e twice.

g. Add 2 mL of 100% ethanol, then mix by vortexing briefly.

h. Incubate for 20 minutes.

i. Centrifuge for 5 minutes at 7500 × g at 4°C.

j. Discard the supernatant with a micropipettor.

k. Air dry the protein pellet for 5–10 minutes.

Solubilize the proteins

a. Resuspend the pellet in 200 µL of 1% SDS by pipetting up and down.
Note: To ensure complete resuspension of the pellet, we recommend that you incubate the sample at 50°C in a water bath or heat block.

b. Centrifuge for 10 minutes at 10,000 × g at 4°C to remove insoluble materials.

c. Transfer the supernatant to a new tube. Proceed directly to downstream applications, or store the sample at –20°C.

Measure protein concentration by Bradford assay. Note: SDS concentration mush be <0.1%.

Proteins Dialyse

1. Load the phenol-ethanol supernatant into the dialysis membrane. Note: The phenol-ethanol solution can dissolve some types of dialysis membranes (cellulose ester, for example). Test dialysis tubing with the membrane to assess compatibility before starting.

2. Dialyze the sample against 3 changes of 0.1% SDS at 4°C. Make the first change of solution after 16 hours, the second change 4 hours later (at 20 hours), and the final change 2 hours later (at 22 hours). Note: A SDS concentration of at least 0.1% is required to resolubilize the proteins from the pellet. If desired, the SDS can be diluted after solubilization.

3. Centrifuge the dialysate for 10 minutes at 10,000 × g at 4°C.

4. Transfer the supernatant containing the proteins to a new tube.

5. (Optional) Solubilize the pellet by adding 100 µL of 1% SDS and 100 µL of 8 M urea. Proceed directly to downstream applications, or store the sample at – 20°C.

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