NZY Total RNA Isolation kit

Catalogue number: MB13402, 50 columns

Description

NZY Total RNA Isolation kit is designed for the easy purification of total RNA of highest integrity (longer than 200 bases) from a variety of sources such as animal and plant tissues, bacteria cells and cell cultures. This method uses a denaturing lysis buffer containing guanidine thiocyanate, which inactivates cellular RNases, to ensure the recovery of intact RNA molecules. Ethanol is added to provide selective binding of total RNA into the silica membrane column and impurities are efficiently washed away. To prevent the contamination with DNA, a DNase I solution is directly added onto the silica membrane of the binding column. High-quality RNA is then eluted in RNase-free water. RNA is ready to use for applications like Reverse Transcriptase (RT) PCR, qPCR*, in vitro* translation or cDNA synthesis.

The NZY Total RNA Isolation kit is optimized to isolate up to 70 µg of RNA/column with an $A_{260/280}$ ratio between 1.9 and 2.1 from up to 30 mg of animal tissue, 100 mg of plant tissue, 1×10^9 bacteria cells or 5×10^6 of cultured cells. We suggest not exceeding the maximum recommended starting material to prevent a reduction in yield and purity.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (15 to 25°C) and are stable till the expiry date.

Before use, add 0.550 mL of RNase-free water to the DNase I vial. DNase I is sensitive to physical denaturation so do not vortex but instead mix gently by inverting the tube. The reconstituted DNase I should be stored at -30°C to -15°C and is stable for 6 months. Add 50 mL of 100% molecular biology grade ethanol to the NWR2 bottle. Buffers NR and NWR1 contain guanidine salts. Wear gloves and goggles when using this kit.

System Components

Component	Volume (50 preps)
Buffer NR	25 mL
Buffer NI	25 mL
Buffer NWR1	15 mL
Buffer NWR2 (concentrate)	12.5 mL
RNase-free Water	15 mL
Digestion buffer	5 mL
DNase I (lyophilized)	1 vial
NZYSpin Homogenization Columns (purple rings)	50
NZYSpin Binding Columns (blue rings)	50
Collection tubes (2 mL)	150
Collection tubes (1.5 mL)	50

Guidelines for using NZY Total RNA Isolation kit

- RNA preparation using NZY Total RNA isolation kit can be performed at room temperature. However, isolated RNA should be treated with care because RNA is very sensitive to trace contaminations of RNases. Be certain not to introduce any RNases during the whole purification process. **Wear gloves always during RNA preparation and change gloves frequently**. To ensure RNA stability store pure RNA at -20 °C for short-term or at -70 °C for long-term.
- Reducing agents: DTT or TCEP (tris(2-carboxyethyl)phosphine) may be used as alternative to β-mercaptoethanol. Use a final concentration of 10-20 mM in Buffer NR.
- We suggest preparing the Digestion Mix before starting the RNA isolation protocol. For each isolation prepare the exact amount of Digestion mixture required as follows:

DNase I (reconstituted)	10 µL
Digestion buffer	90 µL

Mix by gentle pipetting. Store this mixture on ice.

- Optimal amounts of sample material to use in the preparation of RNA using the NZY Total RNA isolation kit are presented in Table 1.

Table 1. Amount of samples material.

Sample	Amount
Tissue	Up to 30 mg
Plant	Up to 100 mg
Cultured cells	Up to 5×10^6 cells
Bacterial cells	Up to 10^9 cells

Protocol for purification of total RNA from tissues or cells

I. Sample preparation

- Animal tissues: Cut up to 30 mg tissue sample (see table 1) into small pieces and place it in a RNase-free microcentrifuge tube. Proceed with step II.
- Plant tissues: Freeze small pieces of plant tissues (see table 1) in liquid nitrogen and grind to a fine powder using a mortar and pestle. Transfer the tissue powder to a RNAse-free microcentrifuge tube. Proceed with step II.
- Cultured cells: Pellet up to 5×10^6 cultured cells (see table 1) at 6,000 x*g* for 5 min at 4 °C. Discard supernatant and add buffer NR directly to cell pellet. Proceed with step II.
- Bacterial cells: Pellet up to 10^9 bacteria cells (see table 1) at 6,000 x*g* for 5 min at 4 °C. Discard the supernatant completely and resuspend the cell pellet in 100 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing 1 mg/mL lysozyme (for Gram-negative strains) or 2 mg/mL lysozyme (for Gram-positive strains). Vortex vigorously and incubate at 37 °C for 10 minutes. Proceed with step II.

  Note: *For Gram-positive bacteria or other microorganisms with extremely resistant cell wall, may be necessary to optimize the conditions of treatment with lysozyme (e.g. incubation time and lysozyme concentration).*

  Homogenization by mechanical disruption may also be performed with bacteria cells using metallic beads. After cell pellet resuspension in 350 µL of buffer NR (reducing agent is not required), add beads and proceed with cells homogenization by bead beating. Centrifuge for 1 min at 11,000 x*g* *to sediment the beads and recover the supernatant. Proceed with step II (2).*

II. Preparation of total RNA

1. Add 350 µL of buffer NR and 3.5 µL β-mercaptoethanol to the cell pellet or to the disrupted tissue. Vortex vigorously.

Note: *DTT or TCEP may be used as alternative to β-mercaptoethanol. Use a final concentration of 10-20 mM DTT within Buffer NR.*
The lysate may be passed through a needle fitted to a syringe to reduce the viscosity.

2. Apply the lysate into a NZYSpin Homogenization column (purple ring) placed in a 2 mL collection tube. Centrifuge for 1 min at 11,000 x*g*. **Save the flow-through**.

Note: *If you need to isolate genomic DNA from the same sample, transfer the flow-through to a gDNA spin column placed in a 2 mL collection tube. Centrifuge for 1 min at 11,000 xg. Use the flow-through for total RNA purification and proceed with the following steps (3-10). For genomic DNA isolation see the support protocol available at www.nzytech.com.*

3. Transfer the flow-through into a new 1.5 mL microcentifuge tube. Add 350 µL of 70% ethanol and mix immediately by pipetting up and down. **Do not centrifuge**.

4. Pipette the lysate and load in a NZYSpin Binding column (blue ring). Centrifuge at 11,000 x*g* for 30 s. Discard the flow-through and place the column into a new collection tube.

5. Add 350 µL of Buffer NI and centrifuge at 11,000 x*g* for 30 s. Discard the flow-through and place the column back into the collection tube.

6. For each isolation, prepare the Digestion Mix in a sterile 1.5 mL microcentrifuge tube (as previously explained). Apply 95 µL of the Digestion Mix directly into the centre of the silica membrane of NZYSpin Binding column (blue ring) and incubate at room temperature for 15 min.

7. Add 200 µL of Buffer NWR1 and centrifuge for 1 min at 11,000 x*g*. Discard the flow-through and place the column in a new collection tube.

8. Add 600 µL of Buffer NWR2 and centrifuge at 11,000 x*g* for 1 min. Discard the flow-through and place the column back in the collection tube.

Note: *Ensure that absolute ethanol was added to Buffer NWR2 before use.*

9. Repeat wash with 250 µL of Buffer NWR2 and centrifuge at 11,000 x*g* for 2 × 1 min to dry the column membrane. Discard the flow-through.

10. Place the NZYSpin Binding Column in a clean 1.5 mL RNase-free microcentrifuge tube. Add 40-60 µL RNase-free water directly to the column membrane. Centrifuge at 11,000 x*g* for 1 min to elute the RNA. Store the RNA at -20 °C for short-term or at -70 °C for long-term.

Quality control assay

Functional assay

All components of NZY Total RNA isolation kit are tested following the isolation protocol described above. The purification system must isolate 50-70 µg of total RNA/column.

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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