MB134_IFU_EN_V2402

NZY Total RNA Isolation Kit

Catalogue number
MB13402

Presentation
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}/A_{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.

Shipping & Storage Conditions

This product is shipped at room temperature. All kit components can be stored at room temperature (15-25 °C) and are stable till the expiry date if stored as specified.

Components

COMPONENT	MB13402 (50 COLUMNS)
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

Reagents, Materials and Equipment Required but Not Provided

- 96-100% ethanol
- 70% ethanol
- 1,5 mL RNAse-free microcentrifuge tubes and disposable tips
- Centrifuge for 1,5 mL microcentrifuge tubes
- Disruption and homogenization equipment's

Specifications

Expected genomic DNA Yield: This protocol was designed for purification up to 70 µg of pure RNA with an A_{260}/A_{280} ratio between 1.9 and 2.1.

RNA Integrity Number (RIN): The integrity of RNA strongly depends on the quality of the sample. RNA isolated from fresh and high-quality samples is expected to have a RIN value ranging between 7 and 9.

Sample material: 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 sample 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

Columns type: silica membrane technology

Elution Volume: 40-60 µL

Standard Protocol

Recommendations before starting

- 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.
- Buffers NR and NWR1 contain guanidine salts. Wear gloves and goggles when using this kit.
- Change gloves frequently.

Procedures before starting

Reagents Preparation

- **DNase I:** 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 -20 °C and is stable for 6 months.
- **Buffer NWR2:** Add 50 mL of ethanol (90-100%) to the NWR2 bottle.
- **Digestion Mix:** For each isolation prepare the exact amount of Digestion mixture required as follows. Then, mix by gentle pipetting and store this mixture on ice.

COMPONENT	VOLUME
DNase I (reconstituted)	10 µL
Digestion Buffer	90 µL

Procedure

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.

Bacteria 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 (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.

Notes:

- *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.

Notes:

- *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 an 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 x g. 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 an 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 microcentifuge 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. You have the flexibility to tailor the elution method and the elution buffer volume to suit your specific application needs:

- **Complete Yields:** To achieve comprehensive yields, perform two elution steps using the volume indicated above, which allows for the retrieval of approximately 90 – 100% of the bound nucleic acids. Afterward, combine the eluates and measure the total yield.
- **Highly Concentrated Eluates:** If your application requires highly concentrated eluates, opt for minimal elution volumes ranging from 40 to 50 µL. This approach typically yields around 60 – 80% of the bound nucleic acids, producing highly concentrated eluates.

Store the RNA at -20 °C for short-term or at -70 °C for long-term.

Quality control 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.

Troubleshooting

LOW OR NO RNA YIELD
• **Sample Material**
Check if the sample was properly stored. Preferentially use fresh samples. If it is not possible freeze your samples in liquid nitrogen and store them at -70 °C. Alternatively, use the RNA Stabilization Solution (Cat. No. MB469) to stabilize and protect RNA, thereby eliminating the need or immediate sample processing.
• **Insufficient Homogenization/Incomplete lysis**
Ensure thorough homogenization of the sample material. Check if the lysate was applied into NZYSpin Homogenization column.

• **Inadequate Buffers preparation**
Check that Buffer NRW2 concentrated was diluted with correct volume of ethanol.
CLOGGED COLUMNS
• **Large amount of sample material**
Check if the amount of starting material used is recommended. Avoid using a large amount of sample; if you do, increase the volume of Buffer NR.
LOW RNA QUALITY
• **Presence of DNA**
Ensure that the Digestion Mix was prepared correctly and applied directly onto the center of the NZYSpin Binding column.
• **Low A_{260}/A_{230} ratio**
The eluate likely contains carry-over of guanidinium thiocyanate. Avoid contaminations throughout the protocol by preventing buffers from coming into contact with the top or lid of the NZYSpin Binding colum.

By addressing these possible causes and implementing the suggested solutions, you can troubleshoot common issues encountered during the RNA isolation process and achieve better results with the NZY Total RNA Isolation kit.

MB134_SUP4_EN_V2401

NZY Total RNA Isolation Kit

Catalogue number	Presentation
MB13402	50 columns

Support Protocol for the isolation of total RNA from up to 5x10^7 Yeast cells

I. Sample preparation

Two alternative protocols can be used in yeast cells homogenization. Users may choose between an enzymatic digestion **(a)** or mechanical homogenization **(b)**.

This depends on the available laboratory equipment and personal preference. Homogenization by enzymatic digestion is only recommended for fresh harvested cells. On the other hand, homogenization by mechanical disruption can be performed with yeast cell pellets, stored at -70°C for several months.

a) Homogenization by enzymatic digestion

1. Centrifuge YPD culture at 5,000 xg for 10 min and harvest 2–5 mL.

2. Resuspend pellet in an appropriate amount of sorbitol/lyticase buffer (50–100 U lyticase or zymolase in 1 M sorbitol/100mM EDTA) and incubate at 30°C for 30 min.

3. Centrifuge at 1,000 xg for 10 min and carefully discard supernatant. Depending on the yeast strain it may be necessary to optimize incubation time and lyticase/zymolase concentration.

Proceed with step 1 of the NZY Total RNA Isolation Kit protocol.

b) Homogenization by mechanical disruption

1. Centrifuge YPD culture at 5,000 xg for 10 min, harvest 2–5mL and wash with ice-cold water.

2. Mix 350 µL Buffer NR and 3.5 µL ß-mercaptoethanol and resuspend the cell pellet. Add glass beads (e.g., 300 mg glass beads, 425–600 µm). Shake samples in a swing-mill at 30 Hz for 15 min.

Proceed with step 2 of the NZY Total RNA Isolation Kit protocol.

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MB134_SUP2_EN_V2401

NZY Total RNA Isolation Kit

Catalogue number	Presentation
MB13402	50 columns

Support Protocol for isolation total RNA from Paraffin Embedded Tissue

I. Sample preparation

Xylene is required as additional reagent.

1. Put 10 mg of tissue into a 1.5 mL microcentrifuge tube. Add 300 µL xylene and incubate 5 min with constant mixing at room temperature.

2. To pellet the tissue, centrifuge at 13,000 rpm for 3 min.

3. Discard the xylene and repeat steps 1 and 2 twice. Totalizing three xylene washes.

4. Add 300 µL of 96% ethanol to the tube and incubate 5 min with constant mixing at room temperature.

5. Centrifuge at 13,000 rpm for 3 min to pellet the tissue and discard the ethanol.

6. Repeat steps **4** and **5**, for a total of two ethanol washes

Continue with step 1 of the standard protocol.

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MB134_SUP2_EN_V2401

NZY Total RNA Isolation Kit

Catalogue number	Presentation
MB13402	50 columns

Support Protocol for isolation total RNA from Biological Fluids

I. Sample preparation

Not necessary.

II. Preparation of total RNA

1. Add 350 µL of buffer NR and 3.5 µL β-mercaptoethanol to 100 µL of sample. Vortex vigorously.

2. Homogenization step: not necessary.

3. Add 350 µL of 70% ethanol and mix immediately by pipetting up and down. **Do not centrifuge**.

Proceed with step 4 of the standard protocol.

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MB134_SUP1_EN_V2401

NZY Total RNA Isolation Kit

Catalogue number	Presentation
MB13402	50 columns

Support Protocol for simultaneous isolation of Genomic DNA and total RNA from the same biological sample

I. Sample preparation

Tissue samples: 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.

Cultured cells: Pellet up to 5×106 cultured cells (see table 1) at 6,000 *xg* 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 109 bacteria cells (see table 1) at 6,000 *xg* for 5 min at 4 °C. Discard the supernatant completely and add buffer NR directly to cell pellet. Proceed with step II.

II. Preparation of genomic DNA and total RNA

1. Add 350 µL of buffer NR and 3.5 µL β-mercaptoethanol to 100 µL of sample. Vortex vigorously.

Note: *The lysate may be passed through a needle fitted to a syringe to reduce the viscosity.*

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

3. Transfer the flow-through to a gDNA spin column (green ring) placed in a 2 mL collection tube. Centrifuge for 1 min at 11,000 xg. Save the flow-through for total RNA purification and proceed with step 3 of the standard RNA isolation protocol.

4. Place the gDNA spin column (green ring) in a new 2 mL collection tube and centrifuge for 1 min at > 11,000 xg. Discard flow-through and place the column in a new collection tube.

Proceed with step 7 of the standard genomic DNA purification protocol.

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