NZY miRNA Isolation & RNA Clean-up Kit

Catalogue number: MB45801, 50 columns

Description

The NZY miRNA Isolation & RNA Clean-up Kit is engineered for the purification of total RNA, including miRNA, from cellular material and may be used for general RNA reaction clean-up, with the optional isolation of protein and DNA. The sample is stabilized after the initial sample lysis in Buffer NMIL, devoid of RNase activity. Mechanical disruption may be required for hard-to-lyse samples. Lysis Buffer NMIL features denaturing salts and β-mercaptoethanol, aiding in sample lysis and stabilization. Unlysed sample remnants are filtered out, reducing lysate viscosity. Concurrently, high molecular weight DNA is sheared, preparing it for a more efficient DNase digest. The addition of ethanol creates optimal binding conditions for RNA and DNA fragments over approximately 200 nt to the NZYSpin RNA Column (blue rings). Meanwhile, smaller RNA and proteins are collected in the flowthrough. This separation ensures superior RNA purity, as DNA and proteins are separately and efficiently removed. If total nucleic acid purification, including DNA, is sought, on-column DNAse digestion is omitted. Otherwise, a desalting step preps the NZYSpin RNA Column for the on-column DNA digest. Meanwhile, Buffer NMIPP is added to the flowthrough to precipitate proteins, which are then removed via filtration through an NZYSpin Protein Removal Column (white ring), leaving only small RNA. This is combined with Buffer NMIB, which adjusts binding conditions for small RNA to the NZYSpin RNA Column (blue rings). After DNA digestion, this mixture is loaded into the NZYSpin RNA Column (blue rings). If separate fractions of small and large RNA are desired, the mixture can also be bound to a second, novel, NZYSpin RNA Column (blue rings). After stringent washing steps, pure RNA is eluted in 30 – 100 µL of RNase-free water and is ready for both standard and advanced downstream applications. For a more detailed protocol, refer to the specific sections in the kit's manual.

Processing of Starting samples

For optimal results, it is best to use the maximum amount of starting material as per **Table 1**, to ensure effective purification of both small and large RNA.

Table 1. General kit specifications.

Parameter	**Quantities**
Sample material	10^7 cultured cells < 30 mg human / animal tissue < 30 mg plant material < 150 µL reaction mix Binding capacity 200 µg (NZYSpin RNA Column)
Typical RNA yields	30 mg mouse liver: 100 µg 30 mg mouse kidney: 35 µg 30 mg mouse spleen: 48 µg 30 mg mouse lung: 27 µg 30 mg mouse heart: 24 µg 30 mg porcine liver: 80 µg 30 mg human brain: 11 µg 10^7 HeLa cells: 100 µg 30 mg wheat leaves: 25 µg

RNA remains susceptible to digestion until it is either flash-frozen or disrupted in the presence of RNase-inhibiting or denaturing agents. For long-term storage, it is advised to flash freeze samples and store them at -70 °C to -80 °C as promptly as possible. Once disrupted, samples can be stored in Lysis Buffer NMIL under various conditions: up to one year at -70 °C to -80 °C, up to six months at -20 °C, up to 24 hours at +2 °C to +8 °C, or several hours at room temperature. When you are ready to isolate RNA from frozen samples in Lysis Buffer NMIL, thaw them slowly until there are no visible salt crystals. During the preparation process, it is crucial to always wear gloves and change them regularly. Only use RNase-free equipment.

A. Processing Cultured tissue and cells

Tissue and cells should be collected through centrifugation. For adherent cells, trypsinization may be necessary prior to collection. Consider the potential for expression profile alterations during trypsinization and extended washing or centrifugation processes.

To perform direct lysis in culture flasks (optional) proceed as follows:

Adherent cells: adherent cells can be lysed directly in the culture flask. After removing the culture medium, wash cells with Phosphate Buffered Saline (PBS) before adding Lysis Buffer NMIL.

Monolayer Cells: Fully remove the culture medium and rinse cells once with PBS. Apply 300 µL Buffer NMIL per 5×10^6 cells directly onto the culture disk and incubate for 5 min at room temperature. Alternatively, following trypsinization, collect up to 10^7 cells via centrifugation, eliminate supernatant, and add 300 µL Buffer NMIL. Lyse cells by pipetting or vortexing.

Suspension Cells: Collect up to 10^7 cells through centrifugation, discard supernatant, and add 300 µL Buffer NMIL. Lyse cells by pipetting or vortexing.

In all methods, transfer precisely 300 µL of lysate to NZYSpin Homogenization Filter Columns (violets ring) placed in a Collection Tube (2 mL, lid).

B. <u>Processing Animal and plant tissue</u>

Animal and plant tissue, protected by a cell wall, often requires mechanical disruption for effective lysis and RNA stabilization with Buffer NMIL. Various mechanical disruptors can assist in breaking down the cells.

Mortar and Pestle: Use this basic but effective technique with prechilled tools and a constant liquid nitrogen supply to prevent thawing. Grind tissue into a fine powder and transfer up to 30 mg into a 1.5 mL centrifuge tube. Add 300 µL Buffer NMIL and vortex vigorously. Transfer the lysate to a NZYSpin Homogenization Filter Column (violets ring) in a 2 mL Collection Tube.

Shearing Force Devices: Load your tissue and 300 µL Buffer NMIL per 30 mg sample into a suitable lysis tube. Disrupt according to the manufacturer's instructions. Transfer the lysate to a NZYSpin Homogenization Filter Column (violets ring).

Regardless of the disruption method, ensure not to exceed 30 mg of tissue per 300 µL Buffer NMIL and refrain from diluting or mixing Buffer NMIL with other fluids. Always transfer exactly 300 µL of lysate to a NZYSpin Homogenization Filter Column (violets ring) in a 2 mL Collection Tube.

C. <u>Processing Bacterial and Yeast</u>

The robust cell walls of bacteria and yeast necessitate the use of lysozyme or lyticase/zymolase solutions, respectively, for efficient breakdown. Alternatively, sonication or mechanical disruption can be employed. To preserve expression profiles, it's crucial to minimize incubation durations.

Elution procedures

The elution volume choice affects RNA recovery and concentration. Balancing high yield and high concentration depends on downstream application requirements. Recommended elution volumes are:

- 30 µL: high concentration, lower total yield
- 50 µL: medium concentration and yield
- 100 µL: high yield, lower concentration

A minimum of 30 µL is required to completely wet the silica. Lower volumes result in significantly less yield. Multiple elution steps, using the first eluate as the elution buffer for the subsequent step, can enhance the total yield. To augment yield and concentration in one step, elution can be performed with RNase-free water heated to 90 °C. However, note that this can introduce larger pipetting errors and variations in the final volume.

Storage conditions and reagents preparation

Lyophilized RNase-free rDNase: Store at +4 °C upon arrival (stable for at least one year).

All other kit components: Store at room temperature (15 – 25 °C). If salt precipitation occurs, warm the bottle at 30 – 40 °C and stir until dissolved.

Prepare Wash Buffer NMIW2 by adding 48 mL of 96 – 100 % ethanol to NMIW2 concentrate, and store at room temperature. Prepare RNase-free rDNase as follows: Add 3 mL of Digestion Buffer to each vial and let it rest for 1 min. Gently swirl to dissolve rDNase without vigorous agitation. Divide rDNase solution into aliquots and store at -20 °C (stable for at least 6 months). Do not freeze/thaw more than three times.

Caution: Buffers NMIL, NMIMD, and NMIW1 contain chaotropic salt. Avoid contact with skin or eyes and never combine with bleach or acidic solutions due to the presence of guanidinium thiocyanate.

Components

Component	Volume (50 preps)
Buffer NMIL	30 mL
Buffer NMIPP	20 mL
Buffer NMIB	60 mL
Buffer NMIMD	25 mL
Buffer NMIW1	35 mL
Buffer NMIW2 (concentrate)	12 mL
RNase-free Water	13 mL
Digestion buffer	7 mL
rDNAse (lyophilized)	2 vials
NZYSpin Homogenization Filter Columns (violets rings)	50
NZYSpin RNA Columns (blue rings)	50
NZYSpin Protein Removal Columns (white rings)	50
Collection tubes (1.5 mL)	50
Collection tubes (2 mL)	50
Collection tubes (2 mL, lid)	50

RNA purification from animal tissue, plant material and cultured cells

Before initiating the protocol, ensure the availability of 96 – 100% ethanol and confirm that rDNase and Wash Buffer NMIW2 have been prepared as specified above.

1. **Cell Lysis:**

   Tissue and plant material: disrupt up to 30 mg of sample in 300 µL Buffer NMIL mechanically. Adjust lysis conditions as per sample type.

   Cultured cells: lyse up to 10^7 cells in 300 µL Buffer NMIL and mix vigorously. Allow a 5-minute incubation at room temperature.

2. **Lysate Homogenization:** Using an NZYSpin Homogenization Filter Column (violet ring) in a 2 mL collection tube, load lysate. Centrifuge for 1 min at 11,000 *x g*. Transfer the supernatant to a fresh tube if a pellet is visible.

3. **Large RNA/DNA Binding Condition Adjustment**: Mix 150 µL of 96 – 100 % ethanol with 300 µL flowthrough, vortex, and allow 5-minute incubation at room temperature. After the addition of ethanol, a precipitate may become visible. Do not remove the precipitate and proceed to step 4.

4. **Binding of Large RNA and DNA**: Load the sample into a NZYSpin RNA Column (blue ring) and centrifuge for 1 min at 11,000 *x g*. Retain both column and flowthrough.

5. **Silica Membrane Desalting**: Rinse the NZYSpin RNA Column (blue ring), inserted in a new Collection Tube (2 mL) without lid, with 350 µL Buffer NMIMD. Then, centrifuge for 1 min at 11,000 *x g*. Discard flowthrough.

6. **DNA Digestion**: Apply 100 µL rDNase solution directly onto the silica membrane. Leave at room temperature for at least 15 minutes, until steps 7 – 10 are completed.

7. **Protein Precipitation**: Add 300 µL Buffer NMIPP to the flowthrough from Step 4, vortex, then centrifuge for 3 min at 11,000 *x g*.

8. **Residual Debris Removal**: Load supernatant into a NZYSpin Protein Removal Column (white ring), and centrifuge for 1 min at 11,000 x g. Discard the column and keep the flowthrough.

9. **Small RNA Binding Condition Adjustment**: Add 800 µL Buffer NMIB to the flowthrough and vortex.

10. **Binding of Small RNA**: Add 600 µL of the mixture into the NZYSpin RNA Column (blue ring) from step 4. *Attention: Do not centrifuge the NZYSpin RNA Column (blue ring) with the rDNase from step 6 before the mixture of step 9 is added.*

    Centrifuge for 30 s at 11,000 *x g*. Repeat twice to load all sample.

    Note: In case you want to separate large and small RNAs use a fresh NZYSpin RNA Column (blue ring) for this step.

11. **Membrane Washing and Drying:**

    - 1st wash: Rinse column with 600 µL Buffer NMIW1, centrifuge for 30 s at 11,000 *x g*.

- 2nd wash: Rinse column with 700 µL Buffer NMiW2, centrifuge for 30 s at 11,000 *x g*.
- 3rd wash: Rinse column with 250 µL Buffer NMiW2, centrifuge for 2 min at 11,000 *x g* to dry the membrane.

12. RNA Elution: Add RNase-free water (30 µL for high concentration, 50 µL for medium, or 100 µL for high yield) to the column. Incubate for 1 min at room temperature, and centrifuge for 30 s at 11,000 *x g*.

RNA clean-up

Before initiating the protocol, ensure the availability of 96 – 100% ethanol and confirm that rDNase and Wash Buffer NMIW2 have been prepared as specified above.

1. **Sample Preparation**: Mix 150 µL of the sample with 150 µL of Buffer NMIL. For volume adjustments, add RNase-free water to achieve 150 µL or increase buffer volumes proportionately for larger samples.
2. **Binding Adjustment for Nucleic Acids (>200 nt)**: Add 200 µL of 96–100% ethanol to the sample, vortex for 5 seconds, and incubate at room temperature for 5 minutes.
3. **Large Nucleic Acid Binding**: Load the sample into a NZYSpin RNA Column (blue ring) placed in a 2 mL collection tube. Centrifuge for 30 seconds at 11,000 *x g*. Retain the flowthrough and column.
4. **(Optional) DNA Digestion**: Add 350 µL of Buffer NMIMD to the column, centrifuge for 1 min, discard flowthrough, and return the column to the collection tube. Then, add 100 µL of rDNase solution directly onto the silica membrane and incubate at room temperature for at least 15 minutes (until step 5 is completed).
5. **Binding Adjustment for Nucleic Acids (<200 nt)**: Mix the flowthrough from step 3 with 100 µL of Buffer NMIPP, vortex for 5 seconds, and incubate for 5 minutes. Then, add 800 µL of Buffer NMIB and vortex for 5 seconds.
6. **Small Nucleic Acid Binding**: Load 700 µL of sample into the column containing large nucleic acids from steps 3 or 4 (in case DNA is digested). Centrifuge for 30 seconds at 11,000 *x g*. Repeat this step to process the remaining sample.

   In case you want to separate large and small RNAs use a fresh NZYSpin RNA Column (blue ring) for this step.
7. **Silica Membrane Wash and Dry**: First, add 700 µL of Buffer NMIW2 to the column and centrifuge for 30 seconds at 11,000 *x g*. Repeat if necessary for optimal A260/230 ratios. For the final wash, add 250 µL of Buffer NMIW2 and centrifuge for 2 min at 11,000 *xg*.
8. **RNA Elution**: Transfer the column to a fresh 1.5 mL collection tube. Add RNase-free water (30 µL for high concentration, 50 µL for medium, or 100 µL for high yield), incubate for 1 minute at room temperature, and then centrifuge for 30 seconds at 11,000 *x g*.

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