NZY Plant/Fungi RNA Isolation Kit

Catalogue number: MB45601, 50 columns

Description

The NZY Plant/Fungi RNA Isolation Kit is engineered to isolate RNA from various plant and fungal materials. It excels even when dealing with samples containing high levels of starch, sugar, secondary metabolites, or any other compounds that could hinder standard RNA isolation protocols. The isolation process initiates with a mechanical disruption of the plant material. This can be achieved by grinding the sample in liquid nitrogen or utilizing any other effective disruption method. Following the disruption, the samples are immersed in a lysis buffer laden with a high concentration of chaotropic ions. This lysis buffer swiftly neutralizes RNases that are ubiquitously present in all biological matter. Plant debris are eliminated after passing through a NZYSpin RNA Plant/Fungi Filter column. A binding solution is then introduced, generating conditions conducive to the absorption of RNA onto the silica membrane. Further, the application of two distinct buffers in washing steps efficiently eradicates salts, metabolites, and macromolecular cellular components. Ultimately, pure RNA with a typical RNA Integrity Number (RIN) of 7-9 is obtained by elution under low ionic strength conditions using RNase-free water. The RNA extraction protocol using the NZY Plant/Fungi RNA Isolation Kit can be executed at ambient temperature. However, the eluate necessitates careful handling due to RNA's inherent sensitivity to trace RNase contamination, typically found on general laboratory ware, fingerprints, and dust. To safeguard RNA stability, it is advised to store the RNA at -20 °C for short-term storage and -70 °C for long-term storage.

The NZY Plant/Fungi RNA Isolation Kit is primarily designed for extracting RNA from a diverse range of plant tissues and organs, and filamentous fungi. Please note that the kit is not designed to isolate small RNA (< 200 nt). A sample input ranging from 50 – 500 mg per preparation is typically recommended. Please refer to Table 1 for comprehensive recommendations. The kit has the capacity to isolate up to 70 µg RNA, making it suitable for an array of downstream applications such as qRT-PCR, cDNA synthesis, Northern blotting, and more.

Handling and general recommendations

Protection against digestion by plant RNase is not conferred to RNA until the sample material is either flash-frozen or disrupted in the presence of RNase-inhibiting or denaturing agents. Consequently, it is essential that samples are processed as swiftly as possible or promptly flash-frozen in liquid nitrogen and stored at -70 °C. In the case of using frozen samples, it is critically important to ensure the samples thaw only during the mechanical disruption in the presence of lysis buffer. Any deviation from this protocol will compromise the RNA quality instantaneously. Plant material that has undergone lysis in Lysis buffer NPFL can be stored at -20 °C for a minimum duration of 2 weeks. During the entire preparation process, it is strongly advised to wear gloves and change them regularly to prevent contamination.

Mechanical disruption is necessary for most plant sample materials, with several options available for this process. The traditional method of sample disruption using a mortar, pestle, and liquid nitrogen is applicable for most sample types. Although this typically results in excellent RNA quality, the RNA yield might be slightly lower compared to methods using bead tubes or extraction bags. The elution method and volume can be adjusted to achieve optimal RNA concentrations for respective downstream applications. Besides the standard method outlined in the individual protocols (recovery rate about 70 – 90 %), possible modifications include:

• High yield: Conduct two elution steps with the volume indicated in the individual protocol. This procedure will typically elute approximately 90 – 100 % of bound nucleic acid.

• High yield and high concentration: Perform elution with the standard elution volume and reapply the eluate onto the column for re-elution.

For optimal stability, keep the eluted RNA immediately on ice post-elution. Short-term storage necessitates freezing at -20 °C, while long-term storage requires freezing at -70 °C.

Storage conditions and reagents preparation

The components of the kit should be maintained at a temperature range of 15 – 25 °C and are confirmed to be stable until the date indicated on the package label. Storage at temperatures lower than the recommended range may result in salt precipitation. During storage, particularly at low temperatures, Buffer NPFN (not provided, MB46101) may form a precipitate. Should this occur, it can be readily dissolved by warming the bottle to 40 °C prior to use.

Before initiating any protocol with the NZY Plant/Fungi RNA Isolation Kit, please prepare the Wash Buffer NPFW2 as follows: add 100 mL of 96 – 100 % ethanol to the Wash Buffer NPFW2 bottle. Once the ethanol has been added, clearly mark the bottle to indicate that this step has been completed. The prepared Wash Buffer NPFW2 can be safely stored at 15 – 25 °C for a minimum duration of one year.

Please be aware that the Buffer NPFL includes guanidine hydrochloride, a compound that can react vigorously when mixed with bleach (sodium hypochlorite). It is imperative that you **DO NOT** add bleach or acidic solutions directly to the waste from sample preparation.

System Components

Component	50 columns
Buffer NPFL	30 mL
Buffer NPFR	5 mL
Buffer NPFB	45 mL
Buffer NPFW1	30 mL
Buffer NPFW2 (concentrate)	25 mL
RNase-free Water	15 mL
NZYSpin RNA Plant/Fungi Filter Columns	50
NZYSpin RNA Plant/Fungi Columns (light blue rings)	50
Collection tubes (2 mL)	150
Collection tubes (1.5 mL)	50

User-Supplied Reagents, Consumables, and Equipment

- 96 – 100% Ethanol (required for Buffer NPFW2 preparation)
- Buffer NPFN (for processing acidic samples). Refer to www.nzytech.com for order details (MB46101)
- Disposable Pipette Tips
- Manual Pipettes
- Centrifuge suitable for microcentrifuge tubes
- Equipment for Sample Disruption and Homogenization
- Personal Protective Equipment: Lab Coat, Gloves, Safety Goggles

Protocols for isolating RNA from plant and fungal material

Before starting the preparation process, please ensure the following:

- Verify that Wash Buffer NPFW2 has been appropriately prepared as per instructions in the section above.
- Note that during storage, especially at cooler temperatures, Buffer NPFN might develop a precipitate. Such precipitates can be readily dissolved by warming the bottle to 40 °C prior to use.

Please, consult **Table 1** below for choosing the optimal protocol, sample amount, and buffer volumes.

Table 1. Recommendations for handling different sample types.

Sample type	Sample amount	Buffer NPFR	Buffer NPFB	Protocol
Samples rich in secondary metabolites				
Grape Vine Leaf	100 mg	50 µL	500 µL	A
Noble Fir	50 mg	20 µL	500 µL	A
Spruce Needle	50 mg	20 µL	500 µL	A
Ginger Rhizome	500 mg	50 µL	500 µL	A
Fruit tissue				
Kiwi	500 mg	20 µL	750 µL	B
Citrus Fruit	500 mg	20 µL	750 µL	B
Apple	500 mg	10 µL	750 µL	B
Grape Berry	500 mg	50 µL	750 µL	A
Blueberry	500 mg	20 µL	500 µL	B
Tomato	500 mg	20 µL	750 µL	A
Leaves				
Tobacco	100 mg	50 µL	500 µL	A
Wheat	100 mg	20 µL	500 µL	A
Maize	100 mg	20 µL	500 µL	A
Arabidopsis	100 mg	20 µL	500 µL	A
Samples with High Starch Content				
Maize Kernel	100 mg	50 µL	500 µL	A
Wheat Kernel	90 mg	20 µL	500 µL	A
Potato Tuber	50 mg	50 µL	500 µL	A
Other Seeds				
Arabidopsis	100 seeds	20 µL	750 µL	A
Alfalfa Seed	50 mg	20 µL	750 µL	A
Cotton Seed	1 seed ~100mg	20 µL	750 µL	A
Roots				
Alfalfa Root	300 mg	10 µL	500 µL	A
Pea Root	180 – 280 mg	20 µL	500 µL	A
Sugar Beet	500 mg	10 µL	500 µL	A
Other Sample Types				
Sugar Cane Stem	500 mg	20 µL	500 µL	A
Fungal Hyphae	50 mg	20 µL	750 µL	B
Fungal Fruiting	50 – 100 mg	10 µL	500 µL	A
Moss	100 mg	10 µL	500 µL	A

Protocol A (RNA isolation from plant and fungal material)

1. Sample Homogenization with Mortar, Pestle, and Liquid Nitrogen

1. Add 500 µL of Buffer NPFL into a 1.5 or 2 mL microcentrifuge tube (not included in the kit).
2. Introduce 10 – 50 µL of Buffer NPFR to the tube (refer to **Table 1** for precise volume).
3. Chill the mortar and pestle using liquid nitrogen or store at -70 °C.
4. Introduce the sample to the chilled mortar containing liquid nitrogen (follow **Table 1** for ideal sample input).
5. Pulverize the sample under liquid nitrogen to obtain a fine powder.
6. Transfer the pulverized sample promptly to the Buffer NPFL/NPFR mix. Ensure the plant material thaws within the lysis buffer.
7. Incubate the lysis tube for 5 minutes at 56 °C. Avoid heat incubation for samples with high starch content (e.g., potato tubers or wheat kernel).
8. Centrifuge at 14,000 *x g* for 1 minute to sediment cell debris. If the debris pellet is not solid enough, extend the centrifuge time (e.g., 3 minutes) and/or speed (e.g., 20,000 *x g*).
9. Proceed with the clear supernatant.

2. Filtration of Lysate

1. Insert a NZYSpin RNA Plant/Fungi Filter Columns into a provided 2 mL Collection Tube.
2. Load the clear lysate from step 1 onto the column.
3. Centrifuge for 1 minute at 14,000 *x g*.
4. If the sample does not completely pass the column, perform an additional centrifuge at 20,000 *x g* for 3 minutes.

3. Adjustment of RNA Binding Conditions

1. Add 500 µL Buffer NPFB to the flow-through and mix by pipetting.
2. Incubate for 5 minutes at room temperature.

4. RNA Binding

1. For each preparation, use one NZYSpin RNA Plant/Fungi Columns (light blue ring) preassembled with a Collection Tube.
2. Load 650 µL of the sample onto the column, then centrifuge for 30 seconds at 14,000 *x g*. Discard the flow-through.
3. Load the remaining sample volume (approximately 200 µL) onto the column, and centrifuge again for 30 seconds at 14,000 *x g*.

5. Washing and Drying of Silica Membrane

1. First wash: Add 500 µL Buffer NPFW1 onto the column, centrifuge for 1 minute at 14,000 *x g*. Discard the flow-through.
2. Second wash: Add 500 µL Buffer NPFW2 onto the column, centrifuge for 1 minute at 14,000 *x g*. Discard the flow-through.
3. Third wash: Add 500 µL Buffer NPFW2 onto the column, centrifuge for 1 minute at 14,000 *x g*. Discard the flow-through.
4. Optional: For certain samples causing discoloration of the silica or the eluate, an additional wash is recommended. Repeat the third wash step.

6. RNA Elution

1. Insert the column into a fresh 1.5 mL Collection Tube (provided).
2. Add 50 µL of RNase-free water onto the column. Let it sit for approximately 1 minute at room temperature before centrifuging for 1 minute at 14,000 *x g*.
3. To achieve higher RNA concentrations, use 40 µL for elution. However, note that overall yield may decrease with smaller volumes.

For further alternative elution procedures see section above.

Protocol B (RNA isolation from acidic samples, e.g., fruits and other samples)

Preparation Pre-check:

- Verify if the Wash Buffer NPFW2 has been appropriately prepared, as directed in the above section.
- Ensure the availability of the Neutralization Buffer NPFN. Refer to www.nzytech.com for order details (MB46101).
- Check **Table 2** for required volumes of Buffer NPFN per sample.

Table 2. Suggested volumes of Buffer NPFN to use per sample with Protocol B.

Sample Type (Fruit Tissue)	Volume of Buffer NPFN per Preparation
Kiwi	50 µL
Lemon	50 µL
Apple	15 µL
Orange	15 µL
Blueberry	50 µL
Fungal Hyphae	0 - 50 µL

1. Sample Homogenization with Mortar, Pestle, and Liquid Nitrogen

1. Begin by pouring 500 µL of Buffer NPFL into a microcentrifuge tube (1.5 or 2 mL size). Please note, this tube is not provided in the kit.

2. Introduce 10 – 50 µL of Buffer NPFR into the same tube. For the optimal volume of Buffer NPFR refer to **Table 1**.
3. Similarly, incorporate 10 – 50 µL of Buffer NPFN to the tube. The recommended volume of Buffer NPFN is available in **Table 2**.
4. Pre-chill a mortar and pestle using liquid nitrogen or by storing it at -70 °C.
5. Add your sample (approximately 500 mg) to the pre-chilled mortar, filled with liquid nitrogen.
6. Proceed to grind the sample within the liquid nitrogen until it achieves a powdery consistency.
7. Quickly transfer the ground sample into the microcentrifuge tube containing the pre-mixed buffers, ensuring immediate mixing. The plant material should only defrost within the lysis buffer.
8. Perform centrifugation for 1 minute at 14,000 *x g* to facilitate sedimentation of the cell debris.

Note: If the cell debris pellet does not appear to be firm enough, consider extending the centrifugation time (e.g., to 3 minutes) and/or increasing the centrifugal force (e.g., to 20,000 *x g*).

9. Carefully transfer the clear supernatant to a new tube (not supplied).

Note: For acidic samples, it is crucial to remove cell debris before proceeding to the heat incubation step.

10. Finally, incubate the lysis tube for 5 minutes at 56 °C.

Continue with Protocol A, step 2: "Filtration of Lysate".

Removal of traces of contaminant DNA

When samples contain a high initial DNA content and are analysed by downstream applications highly sensitive towards DNA contamination, an additional DNA digest might be required. Protocols for DNase treatments are given below. The NZY DNAse I (MB19901) is required for this procedure.

Protocol I: DNA Digestion in Solution

1. **DNA Digestion mixture preparation**: Add 6 µL of Digestion Buffer and 0.6 µL of DNase I to 60 µL of the eluted RNA. Alternatively, mix 100 µL of Digestion Buffer and 10 µL of DNase I and add one-tenth of the volume to the RNA eluate.
2. Incubate for 10 minutes at 37 °C.
3. Use a suitable RNA clean-up procedure to repurify RNA, such as the NZY miRNA Isolation & RNA Clean-up Kit (MB45801), or a procedure that involve ethanol precipitation (see below).

Ethanol Precipitation:

- Add 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 96-100% ethanol to one volume of the sample. Mix thoroughly.
- Incubate for a few minutes to several hours at -20 °C or 4 °C.

Note: Longer incubation times are needed for samples with low RNA concentrations. Shorter times are sufficient for samples with high RNA concentrations.

- Centrifuge for 10 minutes at maximum speed.
- Wash the RNA pellet with 70% ethanol.
- Dry the RNA pellet and re-suspend the RNA in RNase-free water.

Protocol II: On-Column DNA Digestion

1. Proceed with the purification procedure according to Protocol A until you have washed the column with 500 µL Buffer NPFW1 (as in step 5).

- Apply 95 µL of rDNase reaction mixture (10 µL DNAse I + 90 µL DNAse I reaction buffer) directly onto the center of the silica membrane of the column.
- Incubate at room temperature for 15 minutes.
- Continue the procedure from Protocol A, step 5.2, by adding 500 µL of Buffer NPFW2 onto the column.

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