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*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## **Quick-RNA™ Plant Miniprep Kit**

Catalog No. R2024

### Highlights

- Quick, 10 minute isolation of inhibitor-free total RNA (~50 µg) from a wide variety of plant samples using ultra-high density *BashingBeads™* and *Zymo-Spin™* column technologies.
- High-quality RNA eluted in ≥25 µl is ready for reverse transcription, microarray, sequencing, etc.

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For assistance, contact us at [tech@zymoresearch.com](mailto:tech@zymoresearch.com).

## Product Contents

<b>Quick-RNA™ Plant Miniprep Kit (Kit Size)</b>	<b>R2024 (50 preps.)</b>
<b>RNA Lysis Buffer</b>	50 ml
<b>RNA Prep Buffer</b>	25 ml
<b>RNA Wash Buffer<sup>1</sup> (concentrate)</b>	12 ml
<b>DNase/RNase-Free Water</b>	4 ml
<b>Prep Solution</b>	30 ml
<b>ZR BashingBead™ Lysis Tubes (2.0 mm)</b>	50
<b>Zymo-Spin™ IICG Columns</b>	50
<b>Zymo-Spin™ IICR Columns</b>	50
<b>Zymo-Spin™ III-HRC Filters</b>	50
<b>Collection Tubes</b>	2x 50
<b>Instruction Manual</b>	1

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

<sup>1</sup> Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate before use.

## Specifications

- **Sample Types** – Leaves, stems, buds, flowers, fruit, seeds, etc.
- **Format** – Bead beating, spin column
- **Yield** – RNA can be eluted into  $\geq 25$   $\mu$ l, allowing for a highly concentrated sample. Maximum RNA binding capacity of the provided column is  $\sim 50$   $\mu$ g.
- **RNA Purity** – High quality total RNA ( $A_{260}/A_{280} > 1.8$ ,  $A_{260}/A_{230} > 1.8$ ) is recovered. In general, traces of DNA may be present in the eluted RNA fraction. Complete removal of DNA can be accomplished by performing an in-column DNase I digestion (page 4).
- **Compatibility** – Compatible with samples stored in RNAlater™.
- **RNA Storage** - RNA is eluted with RNase-free water and can be stored at  $\leq -70$  °C. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Required Equipment** – Microcentrifuge, vortex, cell disrupter/pulverizer (optional).

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.™ Trademarks of Zymo Research Corporation. Disruptor Genie™ and Vortex Genie™ are trademarks of Scientific Industries, Inc. FastPrep® is a registered trademark of Qbiogene, Inc. RNAlater™ is a trademark of Ambion, Inc. Lightcycler™ is a trademark of the Roche Group.

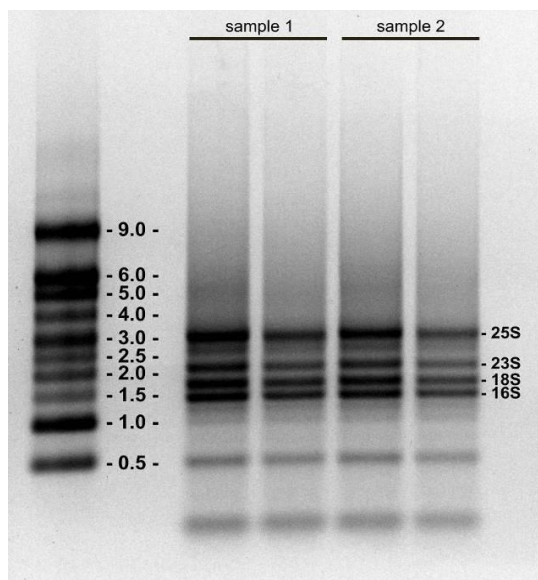
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## Product Description

The **Quick-RNA™ Plant Miniprep Kit** provides for rapid isolation of RNA from various plant samples (*e.g.* leaves, stems, buds, flowers, fruit, seeds *etc.*). For purification of total RNA including small RNAs (~50 µg), the **Quick-RNA™ Plant Miniprep Kit** features a specially formulated **RNA Lysis Buffer**. The **Zymo-Spin™ IICG Column** allows for high-capacity DNA elimination and the subsequent **Zymo-Spin™ ICR Column** efficiently adsorbs total RNA.

The RNA is washed and then eluted with **DNase/RNase-Free Water**. For inhibitor removal, the eluted RNA can be treated by running the sample through the **Zymo-Spin™ III-HRC Filters**. The RNA is suitable for use in various subsequent procedures including RT-PCR.

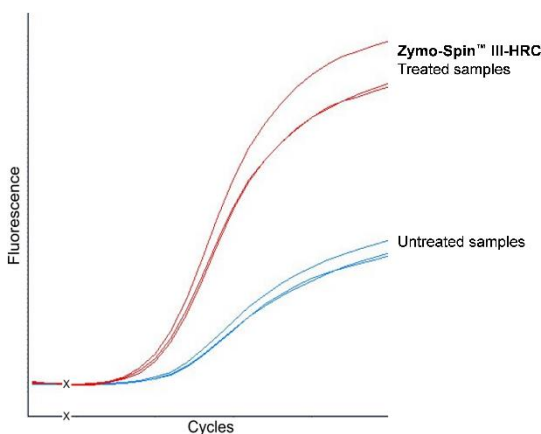
*The entire RNA isolation procedure typically takes about 10 minutes.*



**Figure 1:** Isolation of **total RNA** from **10 mg** of a fresh leaf (*Nicotiana sp.*) using the **Quick-RNA™ Plant Miniprep Kit**. Leaves were minced and processed with a FastPrep®-24 device (*MP Biomedicals*, see page 7). Samples 1 and 2 were loaded in 2x and 1x volume aliquots, respectively, and resolved in a 1% (w/v) nondenaturing agarose gel. RNA Millennium™ Markers (*Ambion*) were used as size standards.

**Figure 2:**

*Nicotiana sp.* leaf samples were spiked with humic acid (*Sigma*) at a final  $A_{230nm} = 0.2$ . Total RNA was isolated with and without the use of the **Zymo-Spin™ III-HRC Filter**. RT-PCR performed with a LightCycler™ 480 (*Roche*) showed an increase in fluorescence signal and detected an early amplification initiation for the **Zymo-Spin™ III-HRC** treated samples compared to the non-treated samples ( $c_p = [30 \text{ vs. } 31]$ , respectively).



For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail [tech@zymoresearch.com](mailto:tech@zymoresearch.com).

### Note:

For isolation of inhibitor-free, PCR-quality DNA from variety of plant and seed samples see **Quick-DNA™ Plant/Seed Miniprep Kit** (Cat. #D6020).

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Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

**Notes:**

<sup>1</sup> For example, up to 150 mg pre-cut leaves of *Nicotiana sp.*

<sup>2</sup> **Sample processing**  
**example:** *Nicotiana sp.*, fresh leaves: 6 m/s, 30-60 seconds – FastPrep<sup>®</sup>-24, or similar. See manufacturer's literature for operating information.

<sup>3</sup> Sample (*i.e.*, *supernatant*) and reagent volumes in this protocol can be adjusted proportionally if needed.

<sup>4</sup> To process samples >800 µl, reload the column.

<sup>5</sup> At this point, RNA samples can be in-column DNase I treated (page 4).

## **Reagent Preparation**

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

## **Protocol**

Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Collect fresh/frozen, finely minced plant sample<sup>1</sup> into a **ZR BashingBead™ Lysis Tube** and add 800 µl **RNA Lysis Buffer**.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process<sup>2</sup>.
3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.
4. Transfer 400 µl of the supernatant<sup>3</sup> into a **Zymo-Spin™ IIICG Column<sup>4</sup>** in a **Collection Tube** and centrifuge for 30 seconds. Save the flow-through!
5. Add 1 volume ethanol (95-100%) to the flow-through in the **Collection Tube** and mix well.
6. Transfer the mixture to a **Zymo-Spin™ IICR Column<sup>4</sup>** in a **Collection Tube** and centrifuge for 30 seconds<sup>5</sup>. Discard the flow-through.
7. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
8. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
9. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
10. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

*Alternatively, for highly concentrated RNA use ≥25 µl elution.*

11. Place a **Zymo-Spin™ III-HRC Filter** in a new **Collection Tube** and add 600 µl **Prep Solution**. Centrifuge at 8,000 x g for 3 minutes.
12. Transfer the eluted RNA (step 10) into a prepared **Zymo-Spin™ III-HRC Filter** in an RNase-free tube (not provided) and centrifuge at exactly 16,000 x g for 3 minutes.

The filtered RNA can be used immediately or stored at -70°C.

## **Appendix A: In-Column DNase Digestion**

The DNase I digestion procedure can be performed using **DNase I Set** (E1010)<sup>1</sup>.

Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Following the RNA binding step (page 3, step 6), prewash the column with 400  $\mu$ l **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (not provided). Mix well by gentle inversion:

<b>DNase I</b>	5 $\mu$ l
<b>DNA Digestion Buffer</b>	75 $\mu$ l

3. Add 80  $\mu$ l of the **DNase I Reaction Mix** directly to the column matrix. Incubate the column at room temperature (20-30°C) for 15 minutes. Then continue with RNA Purification (page 3, step 7).

## **Appendix B: Samples in DNA/RNA Shield™**

1. Bring the lysed sample to at least 800  $\mu$ l with **DNA/RNA Shield™**. Transfer the 800  $\mu$ l sample to a **ZR BashingBead™ Lysis Tube**.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process.
3. Centrifuge the **ZR BashingBead™ Lysis Tube** for 1 minute.
4. Transfer 400  $\mu$ l supernatant to a new tube (not provided).
5. Add 1 volume **RNA Lysis Buffer** to the sample and mix well.
6. Transfer the mixture to a **Zymo-Spin™ IIICG Column** in a **Collection Tube** and centrifuge for 30 seconds. Save the flow-through!
7. Continue with RNA Purification (page 3, step 5).

### **Notes:**

<sup>1</sup> Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

*Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A<sub>260</sub> units/min/ml of reaction mixture at 25°C.*

**Ordering Information**

Product Description	Kit Size	Catalog No.
<b>Quick-RNA™ Plant Miniprep Kit</b>	50 Preps.	R2024

For Individual Sale	Amount	Catalog No.
<b>ZR BashingBead™ Lysis Tubes (2.0 mm)</b>	50	S6003-50
<b>RNA Lysis Buffer</b>	50 ml	R1060-1-50
	100 ml	R1060-1-100
<b>RNA Prep Buffer</b>	10 ml	R1060-2-10
	25 ml	R1060-2-25
<b>RNA Wash Buffer (concentrate)</b>	6 ml	R1003-3-6
	12 ml	R1003-3-12
	24 ml	R1003-3-24
	48 ml	R1003-3-48
<b>Zymo-Spin™ IICR Columns</b>	50	C1078-50
	250	C1078-250
<b>Zymo-Spin™ IICG Columns</b>	50	C1006-50-G
	250	C1006-250-G
<b>OneStep™ PCR Inhibitor Removal Kit</b>	50	D6030
<b>Collection Tubes</b>	50	C1001-50
	500	C1001-500
	1000	C1001-1000
<b>DNase/RNase-Free Water</b>	1 ml	W1001-1
	4 ml	W1001-4
	6 ml	W1001-6
	10 ml	W1001-10



Description	Amount	Cat. No.
<b>Disruptor Genie™, 120V w/ 2 ml tube holder assembly.</b>	1 unit	S6001-2-120
<b>Disruptor Genie™, 240V w/ 2 ml tube holder assembly.</b>	1 unit	S6001-2-240
<b>TurboMix Attachment, 2 ml</b> Permanently mounts to most existing Vortex Genie™ mixers converting them to a Disruptor Genie™.	1 unit	S6004-2

The **Disruptor Genie™** with 2 ml tube holder from Scientific Industries, Inc. (Cat. No. S6001-2 from Zymo Research Corp.)

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