ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Plant Miniprep

RNA from any tough-to-lyse sample

Highlights

- Quick, spin-column purification of total RNA (including small/ microRNAs) from a wide variety of plant samples (e.g., leaves, stems, buds, flowers, fruit, seeds, etc.)
- ZR BashingBead™ Lysis Tubes are ultra-high density, fracture resistant, chemically inert ceramic beads and used for the robust homogenization of any tough-to-lyse sample.
- RNA is ready for Next-Gen Sequencing, RT/qPCR, and any downstream application, etc.

Catalog Numbers:
R2024

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Quick-RNA™ Plant Miniprep	R2024 (50 prep)
RNA Lysis Buffer	50 ml
RNA Prep Buffer	25 ml
RNA Wash Buffer[1] (concentrate)	24 ml
DNase/RNase-Free Water	4 ml
Prep Solution	30 ml
ZR BashingBead™ Lysis Tubes (2.0 mm)	50
Zymo-Spin™ IIICG Columns	50
Zymo-Spin™ IICR Columns	50
Zymo-Spin™ III-HRC Filters	50
Collection Tubes	100
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Sources –** Up to 150 mg plant tissue (e.g., leaves, stems, buds, flowers, fruit, seeds, etc.)
- **Size –** Total RNA including small/microRNAs (≥ 17 nt).
- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- **Binding Capacity – Zymo-Spin™ IICR Column** yield up to 50 µg RNA.
- **Compatibility –** For samples stored in **DNA/RNA Shield™**, see page 6. DNA/RNA Shield™ lyses cells, inactivates nucleases and infectious agents (e.g., virus, pathogens) and is ideal for safe sample storage and transport at ambient temperatures.
- **Elution Volume –** ≥ 25 µl **DNase/RNase-Free Water.**
- **Equipment Needed (user provided) –** Microcentrifuge, vortex, and a high-speed homogenizer/cell disruptor or bead beater (e.g., MP Bio FastPrep-24, Bertin Precellys, etc.) (recommended).

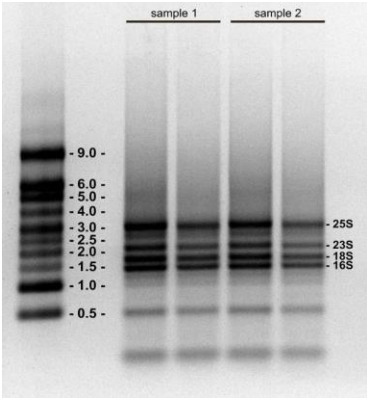
Product Description

The **Quick-RNA™ Plant Miniprep** kit provides for rapid (10-minute) isolation of RNA from various plant samples (e.g. leaves, stems, buds, flowers, fruit, seeds etc.). For purification of total RNA (including small/microRNAs) up to 50 µg.

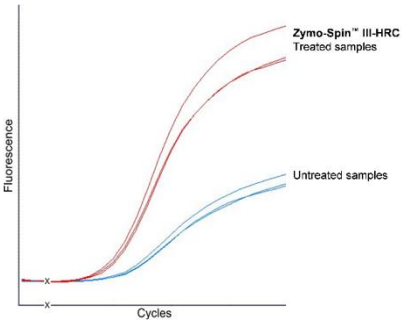
The kit includes unique technology such as the **ZR BashingBead™ Lysis Tubes** and features a specially formulated **RNA Lysis Buffer**. The **Zymo-Spin™ IICG Column** allows for high-capacity DNA elimination and the subsequent **Zymo-Spin™ IICR Column** efficiently adsorbs total RNA.

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**. RNA eluted is suitable for subsequent procedures including RT-qPCR.

Efficient Recovery of RNA from any Plant Sample



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). Samples 1 and 2 were loaded in 2x and 1x volume aliquots, respectively, and resolved in a 1% (w/v) nondenaturing agarose gel. RNA Millenium™ Markers (Ambion) were used as size standards.



Nicotiana sp. leaf samples were spiked with humic acid (Sigma) at a final Ab_{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 vs. 31], respectively).

Protocol

The protocol consists of: (I) Buffer Preparation and (II) Total RNA Purification

(I) Buffer Preparation

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

(II) Total RNA Purification

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

1. Transfer up to 150 mg fresh or frozen, finely minced (pre-cut) plant tissue into a **ZR BashingBead Lysis Tube** (S6003, 2.0 mm) and add 800 µl **RNA Lysis Buffer**.
2. Secure tube in a bead beater fitted with a 2 ml tube hold assembly and process. See example below:

Homogenizers	Bead-Beating Time
High-speed (e.g., MP Bio FastPrep-24, Bertin Precellys)	3 – 5 min
Low-speed (e.g., Vortex Genie)	15 – 20 min

3. Centrifuge the tube for 1 minute to pellet debris.
4. Transfer the cleared supernatant into a **Zymo-Spin™ IIICG Column**[2] (**green**) in a **Collection Tube** and centrifuge. Save the flow-through!
5. To the flow-through, add an equal volume ethanol (95-100%) and mix well.

   Example: Add 400 µl ethanol to 400 µl flow-through.
6. Transfer the mixture into a **Zymo-Spin™ IICR Column**[1] in a **Collection Tube** and centrifuge. Discard the flow-through.

   Optional: At this point, DNase I treatment can be performed. See page 7.
7. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
8. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
9. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).
10. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.
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 the prepared filter in an RNase-free tube (not provided) and centrifuge at 16,000 x g for 3 minutes.

    The eluted RNA can be used immediately or stored frozen.

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1 To process samples > 700 µl, columns may be reloaded.

Appendices

Samples stabilized and stored in DNA/RNA Shield™

✓ If frozen, thaw sample in **DNA/RNA Shield™** to room temperature (20-30°C). Mix well by vortex.

Homogenized Sample

1. Transfer 400 µl of sample homogenized in **DNA/RNA Shield™** to a new RNase-free tube (not provided).
2. Add 400 µl of **RNA Lysis Buffer** (1:1) to the sample homogenized in **DNA/RNA Shield™** and mix well.
3. Proceed to Total RNA Purification (page 5, step 3).

Non-homogenized Sample

1. Transfer 800 µl - 1 ml of sample suspended in **DNA/RNA Shield™** to a **ZR BashingBead Lysis Tube**.
2. Secure the **ZR BashingBead Lysis Tube** in a bead beater fitted with a 2 ml tube holder assembly and process (see homogenization suggestions on page 5, step 2).
3. Centrifuge the **ZR BashingBead Lysis Tube** for 1 minute at high speed (e.g., 16,000 x g).
4. Transfer 400 µl of the supernatant to a new RNase-free tube (not provided).
5. Add 400 µl of **RNA Lysis Buffer** (1:1) to the supernatant and mix well.
6. Proceed to Total RNA Purification (page 5, step 4).

(Appendices continued)

DNase I Treatment (in-column)

- ✓ Perform DNase I treatment with DNase I Set (#E1010) and RNA Wash Buffer (concentrate; #R1003-3-6); available separately.
- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Following RNA binding step (page 5, step 6), add 400 µl **RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** (see table below) in an RNase-free tube (not provided) and mix by gentle inversion. Then add 80 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification (page 5, step 7).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/µl)[1]	5 µl
DNA Digestion Buffer	75 µl

1 Prior to use, reconstitute the lyophilized **DNase I** with 275 µl DNase/RNase-Free Water. Mix by gentle inversion and store frozen aliquots. * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/ml of reaction mixture at 25°C.

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ Plant Miniprep	R2024	50 preps.

Individual Kit Components	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-25 R1060-2-100	25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-24 R1003-3-48	24 ml 48 ml
DNase/RNase-Free Water	W1001-10 W1001-30	10 ml 30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Zymo-Spin™ IICR Columns	C1078-50	50
Zymo-Spin™ III-HRC Filters	C1058-50	50
Collection Tubes	C1001-50	50
OneStep PCR Inhibitor Removal Kit	D6030	50
Prep Solution	D6035-1-30	30 ml
ZR BashingBead™ Lysis Tubes (2.0 mm)	S6003-50	50

Complete Your Workflow

✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

✓ For isolation of RNA from any sample:

Quick-RNA kits	
Miniprep Plus #R1057/R1058	≤ 10^7 cells, ≤ 50 mg tissue
MagBeads #R2132/R2133	Automatable (Tecan, Hamilton, Kingfisher, etc.)

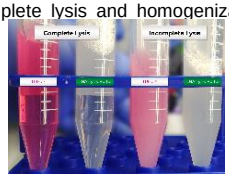
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input: - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image). 
Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	Sample handling: - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. Incomplete lysis and/or cellular debris: - Increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
Low yield	Sample input: - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised nucleic acid recovery. Use less input material and/or increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer. High-protein content: - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.
DNA contamination	To remove DNA: - Perform in-tube DNase I treatment post-purification, refer to the RNA Clean & Concentrator (Cat. R1013) protocol, page 6, "DNase I treatment before RNA clean-up". Then, add 150 µl RNA Lysis Buffer to the 50 µl reaction mix (3:1) and mix well. Add an equal volume ethanol (95-100%) (1:1) and mix well. Proceed to purification step 6, page 5. - In the future, Perform in-column DNase I treatment, step 6, page 5. For future preps, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into DNA/RNA Shield™ and/or RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes

Notes

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