ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR RNA MiniPrep™

Catalog Nos. **R1064 & R1065**

Highlights

- Quick *(15 minute)* RNA isolation *(up to ~25 µg)* from a wide range of sources using *Fast-Spin* column technology.
- RNA eluted into volumes ≥25 µl is suitable for use in RT-PCR and other RNA-based procedures.
- Omits the use of organic denaturants, *β*-mercaptoethanol, and proteases.
- RNA*later*™ compatible.

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Product Contents

ZR RNA MiniPrep™ (Kit Size)	R1064 (50 preps.)	R1065 (200 preps.)	Storage Temperature
RNA Lysis Buffer	50 ml	2x 100 ml	Room Temp.
RNA Prep Buffer	25 ml	4x 25 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	24 ml	3x 24 ml	Room Temp.
DNase/RNase-Free Water	6 ml	10 ml	Room Temp.
Zymo-Spin™ IIIC Columns	50	4x 50	Room Temp.
Zymo-Spin™ IIC Columns	50	4x 50	Room Temp.
Collection Tubes	2x 50	8x 50	-
Instruction Manual	1	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.

[1] Add 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate before use.

Specifications

- **Sample Sources** – Cells from culture or small amounts of solid tissue.
- **Sample Size** – 10^2 to 10^7 cells in suspension or solid form.
- **RNA Recovery** – RNA can be eluted into small volumes, ≥25 µl, allowing for a highly concentrated sample. Maximum RNA binding capacity of provided column is ~25 µg.
- **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. Trace DNA can be removed by DNase digestion (see **Appendices A** and **B** for details).
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.

Product Description

The **ZR RNA MiniPrep™** provides a quick method for high quality total RNA isolation from small amounts of cells and tissue.

The **ZR RNA MiniPrep™** isolates both large and small RNA species without the use of phenol or reducing agents. Small RNAs (*e.g.,* tRNAs, microRNAs) can be recovered following a simple adjustment within the RNA isolation protocol – no extra steps are required!

RNA *(~25 µg)* from 10^2 to 10^7 cells can be eluted into volumes as little as 25 µl in less than 15 minutes.

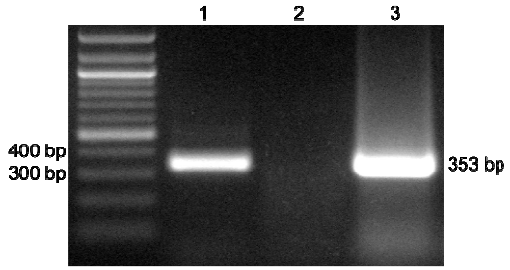


Figure 1:
RNA from human epithelial cells (HCT 116) isolated using the **ZR RNA Prep™** – PCR amplification of *β*-actin transcript post-RT (353bp fragment shown):
1 RT-PCR,
2 PCR negative control (RNA template),
3 PCR positive control (DNA template).

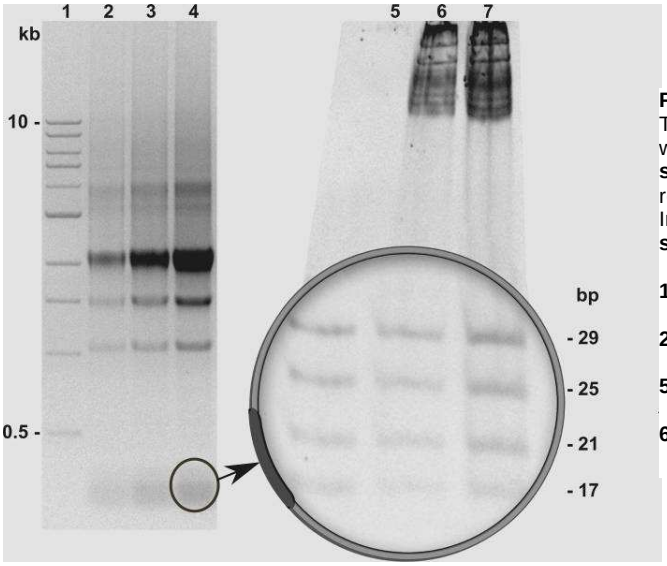


Figure 2:
Total RNA isolated using the **ZR RNA Prep™** was separated in an agarose gel (2-4) and **small RNAs** from the same sample were also resolved in a native polyacrylamide gel (6-7). Input was 10^5 yeast cells spiked with 1µg **ZR small-RNA™ ladder** (Cat. #**R1090**).

1 **ZR 1kb DNA Marker** (Cat. #**M5003, M5006**) *[agarose gel]*
2-4 2, 4, 9 µg total RNA (yeast) + **ZR small-RNA™ ladder** mix *[agarose gel]*
5 **ZR small-RNA™ ladder** (17-29bp ssRNA oligos) *[PAGE]*
6-7 300, 600 ng **ZR small-RNA™ ladder** isolated with **ZR RNA Prep™** *[PAGE]*

The **ZR RNA MiniPrep™** can be used to isolate total RNA from *hard-to-lyse* samples with the **RNA Lysis Buffer** and the ultra-high density **ZR BashingBeads™** coupled with high-speed cell disrupters (e.g., FastPrep®-24, page 7) or to purify RNA directly from mixed DNA/RNA samples (e.g., *in vitro* transcription/translation).

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

Note:

For recovery of small RNAs separated in polyacrylamide gels the **ZR small-RNA™ PAGE Recovery Kit** (Cat. **#R1070**) can be used.

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

Buffer Preparation

Before starting, add 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Protocol[1]

1. Cell homogenization/sample preparation[2]:
   a. **Cell samples:** Isolate cells by gentle centrifugation and remove the supernatant. Resuspend the pelleted cells in 400 µl **RNA Lysis Buffer**.
   b. **Tissue samples:** Add 400 µl **RNA Lysis Buffer** directly to the sample and mechanically homogenize up to 25 mg fresh or frozen tissue.
   c. **Liquid samples/suspensions:** Add 4 volumes **RNA Lysis Buffer** to the sample and mix well *(e.g., 320 µl buffer added to 80 µl sample). Adjust the reagents volume proportionally as needed.*
2. Centrifuge the sample mixture at ≥12,000 × *g* for 1 minute
3. Transfer the lysate (i.e., the supernatant from Step 2) to a **Zymo-Spin™ IIIC Column** in a **Collection Tube**. Centrifuge at 8,000 × *g* for 30 seconds. *Save the flow-through!*
4. Add 0.8 volume ethanol (95-100%) to the flow-through in the **Collection Tube** and mix well *(e.g., 320 µl ethanol added to 400 µl flow-through)*. *For quantitative small RNA recovery, use 2 volumes ethanol (95-100%)*[3].
5. Transfer the mixture to a **Zymo-Spin™ IIC Column**[4] in a **Collection Tube**. Centrifuge at ≥12,000 × *g* for 1 minute[5]. Discard the flow-through.
6. Add 400 µl **RNA Prep Buffer** to the column. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through and replace the **Zymo-Spin™ IIC Column** back into the **Collection Tube**.
7. Add 800 µl **RNA Wash Buffer** to the column. Centrifuge at ≥12,000 × *g* for 30 seconds. Discard the flow-through and place the **Zymo-Spin™ IIC Column** back into the **Collection Tube**. Repeat the wash step with 400 µl **RNA Wash Buffer**.
8. Centrifuge **Zymo-Spin™ IIC Column** at ≥12,000 × *g* for 2 minutes in the emptied **Collection Tube** to ensure complete removal of the wash buffer.
9. Place the **Zymo-Spin™ IIC Column** into an RNase-free tube. Add ≥25 µl **DNase/RNase-Free Water** directly to the column matrix and let stand at room temperature for 1 minute.
10. Centrifuge at 10,000 × *g* for 30 seconds to elute the RNA from the column. RNA can be used immediately or stored at ≤-70 °C (see **Specifications**, page 1).

Notes:

[1] The kit is designed for efficient isolation of total RNA from $1x10^2$ to $1x10^7$ cells.

[2] **ZR RNA MIcroPrep™** is compatible with **RNA*later*™**.

[3] Maximum loading volume for **Zymo-Spin™ IIIC and IIC Column** is 800 µl. Column has to be reloaded to process volumes >800 µl.

[4] The maximum binding capacity of the **Zymo-Spin™ IIC Column** is ~25 µg of RNA.

[5] To perform a **DNase Digestion** following the Step 5 of this protocol, see **Appendix A** and **B** on page 5 and 6.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR RNA MiniPrep™	R1064 R1065	50 Preps. 200 Preps.
ZR RNA MicroPrep™	R1060 R1061	50 Preps. 200 Preps.

For Individual Sale	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25	10 ml 25 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
DNase/RNase-Free Water	W1001-1 W1001-6 W1001-10	1 ml 6 ml 10 ml
Zymo-Spin™ IIIC Columns	C1006-50 C1006-250	50 250
Zymo-Spin™ IIC Columns	C1011-50 C1011-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

Fast-Spin column technology efficiently removes the majority of DNA during RNA purification and is satisfactory for most RNA-based applications. However, if necessary, complete removal of DNA can be achieved by performing a DNase I digestion.

Appendix A

In-Column DNase Digestion

The DNase digestion procedure can be performed using any source of RNase-free DNase I together with its 10X reaction buffer (*e.g.*, 100 U **RNase-free DNase I (1 U/µl) w/ 10X Reaction Buffer** – Zymo Research Cat. No. **E1007**). DNase I maintain activity in the **RNA Wash Buffer** provided in this kit.

1. Make the following DNase I cocktail (for each sample to be treated):

RNase-Free DNase I	10 µl (1 U/µl)
10X Reaction Buffer	10 µl
RNA Wash Buffer	80 µl

2. Following Step 5 of the RNA isolation protocol[1], add 400 µl **RNA Wash Buffer** to the **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge at ≥12,000 x *g* for 30 seconds. Discard the flow through.

3. Add 100 µl DNase I cocktail from Step 1 above directly to the matrix of the **Zymo-Spin™ IIC Column**. Keep the **Zymo-Spin™ IIC Column** in the **Collection Tube**.

4. Incubate the column at 25-37ºC for ≥15 minutes[2], then centrifuge ≥12,000 x *g* for 30 seconds. Discard the flow-through.

5. Continue with Step 6 of the RNA isolation protocol[3].

Notes:

[1] See page 3, Protocol – step 5.

[2] The temperature optimum for DNase I activity is at 37 ºC. An optimal incubation time may vary.

[3] See page 3, Protocol – step 6.

Appendix B

In-Tube DNase Digestion

The procedure can be performed using any source of RNase-free DNase I together with its 10X reaction buffer (*e.g.*, 100 U **RNase-Free DNase I (1 U/µl) w/ 10x Reaction Buffer** – Zymo Research Cat. No. **E1007**).

1. Make the following DNase I cocktail (for each sample to be treated):

RNase-Free DNase I	10 µl (1 U/µl)
10X Reaction Buffer	10 µl
DNase/RNase-Free Water	80 µl

2. Following Step 5 in the RNA isolation protocol[1], add 400 µl **RNA Wash Buffer** to the **Zymo-Spin™ IIC Column** and centrifuge at ≥12,000 x *g* for 30 seconds.

3. Transfer the **Zymo-Spin™ IIC Column** into an RNase-free tube.

4. Add 100 µl DNase I cocktail from Step 1 above directly to the matrix of the column and centrifuge at 500 x *g* 30 seconds. Keep the **Zymo-Spin™ IIC Column** in the RNase-free tube.
   Save the column and the flow-through in the RNase-free tube!

5. Incubate at room temperature for ≥15 minutes[2], then centrifuge ≥12,000 x *g* for 30 seconds.

6. Transfer the **Zymo-Spin™ IIC Column** into a new **Collection tube**.

7. Add 300 µl **RNA Lysis Buffer** to the 100 µl flow-through in the RNase-free tube (from Step 5) and mix well by pipetting.

8. Add 400 µl ethanol (95-100%) to the mixture from Step 7. Mix well by pipetting and reload onto the **Zymo-Spin™ IIC Column** in a **Collection Tube**. Centrifuge at ≥12,000 x *g* for 30 seconds.

9. Continue with Step 6 of the RNA isolation protocol[3].

Notes:

[1] See page 3, step 5.

[2] The temperature optimum for DNase I activity is at 37 ºC. An optimal incubation time may vary.

[3] See page 3, step 6.

The Ultimate Combination For Efficient Sample Lysis!

BashingBead™ Kits From Zymo Research & The FastPrep®-24 Instrument From MP Biomedicals.

Lyse Any Tough or Frozen Sample in 40 Seconds or Less!

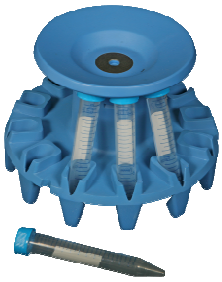
Description	Cat. No.	Amount
FastPrep®-24 Instrument (Supplied w/ 24 x 2 ml head adapter)	S6005	1 unit

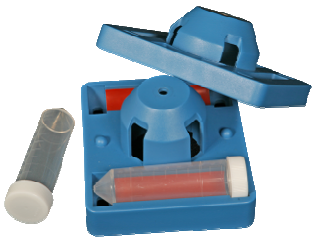
FastPrep® Accessories

For -80°C Lysis

Description	Cat. No.	Amount
HiPrep™ Attachment (Accommodates 48 x 2 ml tubes)	S6005-1	1 unit

Description	Cat. No.	Amount
CryoPrep™ Attachment (Accommodates 24 x 2 ml tubes)	S6005-2	1 unit





Description	Cat. No.	Amount
TeenPrep™ Attachment (Accommodates 12 x 15 ml tubes)	S6005-3	1 unit

Description	Cat. No.	Amount
BigPrep™ Attachment (Accommodates 2 x 50 ml tubes)	S6005-4	1 unit