ZYMO RESEARCH

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

# ZR small-RNA™ PAGE Recovery Kit

Catalog No. **R1070**

## Highlights

- Quick *(45 minute)* recovery of small RNA fragments from polyacrylamide gels.
- *Fast-Spin* column technology allows RNA to be eluted into minimal volumes (≥ 6 µl).
- Eluted RNA is ultra clean and ready for subsequent analysis and molecular manipulation.

## 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 small-RNA™ PAGE Recovery Kit (Kit Size) | R1070 (20 preps.) | Storage Temperature |
|---|---|---|
| **RNA Recovery Buffer** | 10 ml | Room Temp. |
| **RNA MAX Buffer** | 20 ml | Room Temp. |
| **RNA Prep Buffer** | 10 ml | Room Temp. |
| **RNA Wash Buffer**[1] (concentrate) | 6 ml | Room Temp. |
| **Zymo-Spin™ IV Columns** (orange caps) | 20 | Room Temp. |
| **Zymo-Spin™ IIIC Columns** | 20 | Room Temp. |
| **Zymo-Spin™ IC Columns** (with Collection Tubes) | 2x 10 | Room Temp. |
| **Squisher™-Single** | 2x 10 | - |
| **Collection Tubes** | 50 | - |
| **Instruction Manual** | 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 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate (R1070) before use.

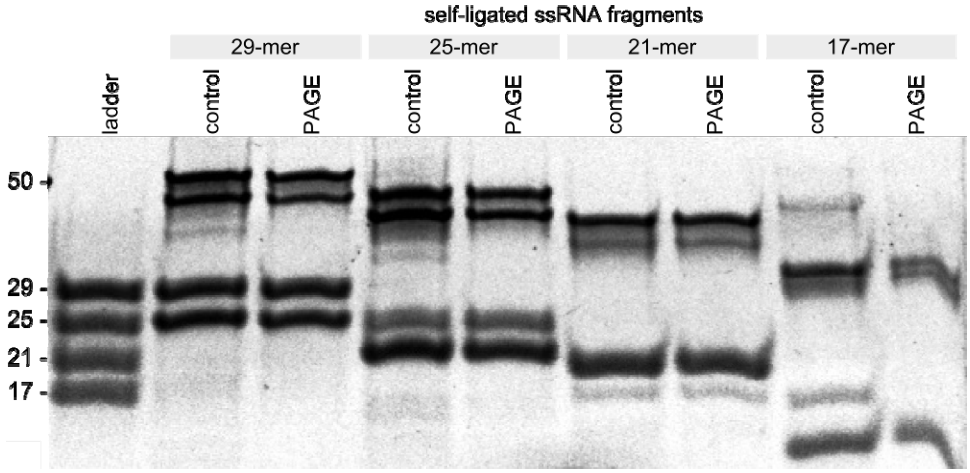
## Specifications

- **Sample Sources** – Single- or double-stranded RNA fragments (17-200 nucleotides) resolved in polyacrylamide gels (tested up to 25% (w/v) polyacrylamide) stained with ethidium bromide or ssRNA-specific dyes (e.g. GelStar®).
- **Format** – Spin column.
- **RNA Purity** - High quality RNA ($A_{260}/A_{280}$ >1.8, $A_{260}/A_{230}$ >1.8) suitable for all downstream RNA-based manipulations.
- **RNA Recovery** – The recovery rate for fragments 17-28 nucleotides is ≥50%. Total binding capacity of the supplied **Zymo-Spin IC™ Columns** is ≥5 µg.
- **Equipment Needed** – Microcentrifuge, 37-65ºC heat source, dry ice or -80ºC freezer.

## Product Description

The **ZR small-RNA™ PAGE Recovery Kit** provides an easy and efficient method for the rapid purification of high quality small RNAs in less than 45 minutes.

The **ZR small-RNA™ PAGE Recovery Kit** is a refinement of the "crush & soak" method that incorporates a unique buffer system together with *Fast-Spin* column technology for improved recovery and added convenience. The recovered RNA can be concentrated at elution step in volumes as small as 6 µl and is ideal for any downstream enzymatic reaction or manipulation.




ladder = ZR small RNA ladder (Cat. #R1090)
control = ssRNA oligo ligation control
PAGE = recovered ssRNA oligo self-ligated

**Recovery and ligation of single-stranded RNA oligonucleotides.** In the image above, the RNA fragments were recovered from a 17.5% (w/v) native polyacrylamide gel using the **ZR small-RNA™ PAGE Recovery Kit**. All fragments shown were resolved in a native PAGE gel following ligation. T4 polynucleotide kinase and T4 RNA ligase I (New England Biolabs, Inc.) were used for the phosphorylation and subsequent ligation of the ssRNA samples. RNA in the gel was visualized with GelStar® Stain (Lonza Rockland, Inc.).

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

## Protocol

*Follow general guidelines to ensure the RNA isolation procedure is performed in an RNase-free environment.*

1. Excise an RNA fragment from a PAGE gel and transfer the slice into a **Zymo-Spin™ IV Column** in a **Collection Tube.**

2. Crush the gel slice with a **Squisher™-Single** against the side of the column. Add 400 µl **RNA Recovery Buffer** directly into the column. Cap the column and incubate at 65°C for 15 minutes.

3. Quick freeze the samples on dry ice or in a -80ºC freezer for 5 minutes, then transfer columns back into 65°C for 5 minutes to thaw.

4. Snap off the **Zymo-Spin™ IV Column** tip and place the column back into a **Collection Tube**. Centrifuge at ≥1,500 × *g* for 30 seconds. Save the flow-through.

5. Transfer the flow-through from the Step 4 to a **Zymo-Spin™ IIIC Column** in a **Collection Tube** and centrifuge at ≥1,500 × *g* for 30 seconds. Save the flow-through.

6. Add 2 volumes of **RNA MAX Buffer** to the flow-through from Step 5 and mix well.

7. Transfer the mixture to a **Zymo-Spin™ IC Column** in a **Collection Tube**. Centrifuge at ≥12,000 × *g* for 30 seconds. Discard the flow-through and place the **Zymo-Spin™ IC Column** back into the **Collection Tube**.

8. Add 400 µl **RNA Prep Buffer** to the column. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through and place the **Zymo-Spin™ IC Column** back into the **Collection Tube**.

9. 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™ IC Column** back into the **Collection Tube**.

10. Repeat Step 9 with 400 µl **RNA Wash Buffer**.

11. Centrifuge the **Zymo-Spin™ IC Column** at ≥12,000 × *g* for 2 minutes in an empty **Collection Tube** to ensure complete removal of the wash buffer.

12. Place the **Zymo-Spin™ IC Column** into a provided **DNase/RNase-Free Tube**. Add 6-15 µl of the provided **DNase/RNase-Free Water** directly to the column matrix and let stand at room temperature for 1 minute.

13. Centrifuge the **Zymo-Spin™ IC Column** at 10,000 × *g* for 1 minute to elute RNA. Recovered RNA can be used immediately or stored at ≤-70 °C.

## Ordering Information

| Product Description | Catalog No. | Kit Size |
|---|---|---|
| **ZR small-RNA™ PAGE Recovery Kit** | R1070 | 20 Preps |

| For Individual Sale | Catalog No. | Amount |
|---|---|---|
| **RNA Recovery Buffer** | R1070-1-10 | 10 ml |
| **RNA MAX Buffer** | R1070-2-20 | 20 ml |
| **RNA Prep Buffer** | R1060-2-10<br>R1060-2-25 | 10 ml<br>25 ml |
| **RNA Wash Buffer** (concentrate) | R1003-3-6<br>R1003-3-12<br>R1003-3-24<br>R1003-3-48 | 6 ml<br>12 ml<br>24 ml<br>48 ml |
| **Zymo-Spin™ IC Columns** | C1004-50<br>C1004-250 | 50<br>250 |
| **Zymo-Spin™ IIIC Columns** | C1006-50<br>C1006-250 | 50<br>250 |
| **Zymo-Spin™ IV Columns** | C1007-50<br>C1007-250 | 50<br>250 |
| **Collection Tubes** | C1001-50<br>C1001-500<br>C1001-1000 | 50<br>500<br>1000 |
| **DNase/RNase-Free Water** | W1001-1<br>W1001-4<br>W1001-6<br>W1001-10 | 1 ml<br>4 ml<br>6 ml<br>10 ml |