Innovation to support science....

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 (and short ss- or dsDNA) fragments from polyacrylamide gels.
- *Zymo-Spin*™ column technology allows nucleic acids to be eluted into minimal volumes (≥ 6 µl) and is ready for subsequent analysis and molecular manipulation.

Contents

ZYMO RESEARCH CORP.

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

For assistance, contact us at tech@zymoresearch.com.

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.
DNase/RNase-Free Water	1 ml	Room Temp.
Zymo-Spin™ IV Columns (orange caps)	20	Room Temp.
Zymo-Spin™ IIICG Columns	20	Room Temp.
Zymo-Spin™ IC Columns (with Collection Tubes)	20	Room Temp.
Squisher™-Single	20	-
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] Before starting, add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Types** – Single- or double-stranded RNA (and DNA) 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.
- **Yield** – The recovery rate for fragments 17 to 28 nucleotides is ≥50 %. Total binding capacity of the supplied **Zymo-Spin IC™ Columns** is ≥10 µg.
- **Required Equipment** – Microcentrifuge, 37 to 65 ºC heat source, dry ice or -80 ºC freezer.

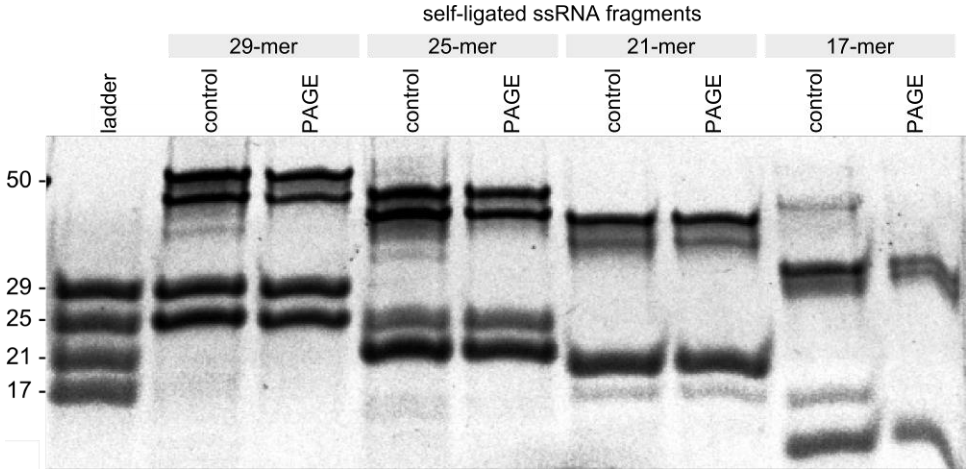
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Product Description

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

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 *Zymo-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.).

Ensure the RNA isolation procedure is performed in an RNase-free environment.

Reagent Preparation

✓ Before starting, add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Protocol

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

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 65ºC for 15 minutes.
3. Quick freeze the samples on dry ice or in a -80ºC freezer for 5 minutes, then transfer columns back into 65ºC 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™ IIICG 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 ≤-70ºC.

Ordering Information

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

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

RNA MADE SIMPLE

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