ZYMO RESEARCH

RNA Purification Made Simple™

ZR small-RNA™ PAGE Recovery Kit

Clean-up RNA from polyacrylamide gels

Highlights

- Quick, 45-minute method for the recovery of purified RNA fragments from polyacrylamide gels.
- Ultra-pure RNA is ≥ 6 µl and is ready for subsequent analysis and molecular manipulation.

Catalog Numbers:
R1070

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

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Product Contents

ZR small-RNA™ PAGE Recovery Kit	R1070 (20 prep)
RNA Recovery Buffer	10 ml
RNA Max Buffer	20 ml
RNA Prep Buffer	10 ml
RNA Wash Buffer (concentrate)	6 ml
DNase/RNase-Free Water	1 ml
Zymo-Spin™ IV Columns (orange caps)	20
Zymo-Spin™ IIICG Columns	20
Zymo-Spin™ IC Columns (with Collection Tubes)	20
Squisher™-Single	20
Collection Tubes	50
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Before starting, add 24 ml 100% ethanol (26 ml of 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Sources –** Single- or double-stranded RNA (and DNA) fragments (17-200 nucleotides) resolved in polyacrylamide gels (up to 25% (w/v) polyacrylamide), also compatible with TBE-, denatured, urea PAGE gels. Compatible with PAGE gels stained with ethidium bromide or ssRNA-specific dyes (e.g., GelStar).

- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. RNA is ready for all downstream manipulations.

- **Recovery –** For 17-28 nt, the recovery rate is ≥ 50%.

- **Binding Capacity –** 10 µg RNA (**Zymo-Spin™ IC Column**).

- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water**.

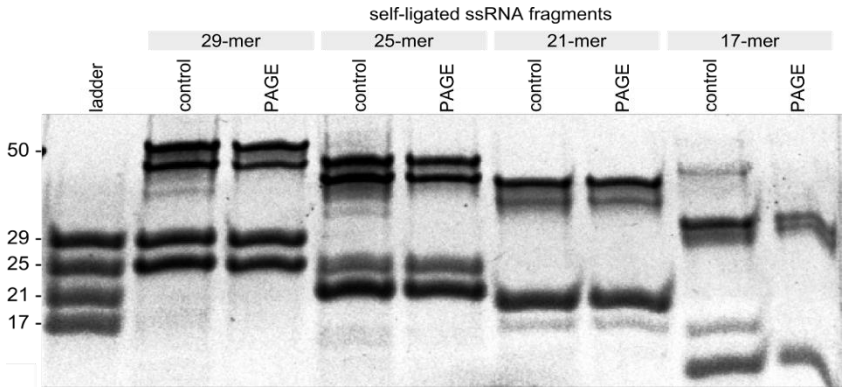
- **Equipment Needed** (user provided) **–** Microcentrifuge, heat source (37-65°C), dry ice or -80°C freezer.

Product Description

The **ZR small-RNA™ PAGE Recovery Kit** provides an easy and efficient method for the rapid purification of high-quality smallRNAs 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.

High-quality Recovery and Ligation of Single-Stranded RNA Oligonucleotides



ladder = ZR small RNA Ladder (Zymo Research; #R1090)
control = ssRNA oligo ligation control
PAGE = recovered ssRNA oligo self-ligated

RNA fragments were resolved in a 17.5% (w/v) native polyacrylamide gel following ligation and recovered using the **ZR small-RNA™ PAGE Recovery Kit**. 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 was visualized with GelStar® Stain (Lonza Rockland, Inc.).

Protocol

The protocol consists of:
(I) Buffer Preparation and (II) RNA Clean-Up

(I) Buffer Preparation

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

(II) RNA Clean-up

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

1. With a scalpel or razor blade, excise the RNA fragment from the PAGE gel[1] and transfer it 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.
3. Add 400 µl **RNA Recovery Buffer** into the column, cap and incubate at 65°C for 15 minutes.
4. Quick freeze the samples on dry ice or in a -80°C freezer for 5 minutes. Then transfer the column back into 65°C for 5 minutes to thaw.
5. Snap off the tip of the column and place into a **Collection Tube** and centrifuge at 1,500 x g for 30 seconds. Save the flow-through!
6. Transfer the flow-through (step 5), into a **Zymo-Spin™ IIICG Column**[2] in a **Collection Tube** and centrifuge at 1,500 x g for 30 seconds. Save the flow-through!
7. To the flow-through (step 6), add 2 volumes of **RNA Max Buffer** (2:1) and mix well.
8. Transfer the mixture to the **Zymo-Spin™ IC Column**[2] in a **Collection Tube** and centrifuge. Discard the flow-through.
9. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
11. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 1 minute ensure complete removal of the wash buffer. Carefully, transfer the column into a nuclease-free tube (not provided).
12. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

    Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

    The eluted RNA can be used immediately or stored frozen.

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1 The amount of polyacrylamide gel excised should be as small as possible.
2 To process samples > 700 µl, **Zymo-Spin™** columns may be reloaded.

Ordering Information

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

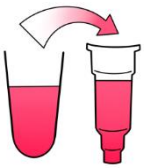
Individual Kit Components	Catalog No.	Amount
RNA Recovery Buffer	R1070-1-10	10 ml
RNA Max Buffer	R1070-2-20	20 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25	10 ml 25 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12	6 ml 12 ml
Zymo-Spin™ IV Columns	C1007-50	50
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Zymo-Spin™ IC Columns	C1004-50	50
DNase/RNase-Free Water	W1001-1 W1001-4	1 ml 4 ml
Collection Tubes	C1001-50	50
Squisher-Single	H1001-50	50

Complete Your Workflow

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

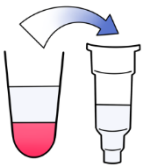
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

✓ The only **direct**, high-throughput and automatable RNA purification from sample lysates in TRIzol (DNase I Set included with all formats):



Direct-zol RNA kits	
Microprep #R2060-R2063	From 1 cell and up
Miniprep #R2050-R2053	Up to 50 ug RNA
Miniprep Plus #R2070-R2073	Up to 100 ug RNA
96-well #R2054-R2057	Spin-plate
MagBeads #R2100-R2105	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):



RNA Clean & Concentrator kit	
#R1013-R1014	DNase I Set included

✓ For NGS:

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

Notes

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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.

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.

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