



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-Duet™ DNA/RNA MiniPrep

Catalog No. **D7001**

Highlights

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

Contents

Product Contents.....	1
Specifications	1
Product Description	2
Buffer Preparation	3
Protocol	3, 4
Appendix	5
Ordering Information	6
Related Products.....	7

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- <i>Duet</i> [™] DNA/RNA MiniPrep (Kit Size)	D7001 (50 preps.)	Storage Temperature
DNA/RNA Lysis Buffer	50 ml	Room Temp.
DNA Prep Buffer	12 ml	Room Temp.
DNA Pre-Wash Buffer	15 ml	Room Temp.
g-DNA Wash Buffer	50 ml	Room Temp.
RNA Prep Buffer	25 ml	Room Temp.
RNA Wash Buffer ¹ (concentrate)	12 ml	Room Temp.
DNase/RNase-Free Water	10 ml	Room Temp.
Zymo-Spin [™] IIC Columns	50	Room Temp.
Zymo-Spin [™] IIIC Columns	50	Room Temp.
Collection Tubes	3x 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.

Specifications

- **Sample Sources** – Cells, small amounts of *easy-to-lyse* tissue, buffy coat, buccal cells, plasma, serum, and other biological liquids. *Not compatible with whole blood.*²
- **Sample Size** – 10² to 5x10⁶ cells in suspension or as tissue.
- **Recovery** – DNA and RNA can be eluted into small volumes (≥25 µl) allowing for a highly concentrated sample. Maximum DNA/RNA binding capacity of the provided columns is ~25 µg.
- **Size Limits** – Capable of recovering genomic DNA up to and above 40 kb. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered. Total RNA ≥17 nucleotides can be recovered.
- **Purity** – High quality genomic DNA and total RNA ($A_{260}/A_{280} >1.8$, $A_{260}/A_{230} >1.8$) is recovered. Traces of DNA may be present in the eluted RNA fraction. Trace DNA can be removed by DNase digestion (see **Appendix**).
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 °C. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge

Note - [™] Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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. RNA*later*[™] is a trademark of Ambion, Inc., Austin, Texas and is protected by various U.S. and foreign patents.

Notes:

¹ Add 48 ml 100% ethanol (52 ml of 95% ethanol) to the 12 ml RNA Wash Buffer concentrate before use.

² For purification of DNA and RNA from whole blood, see the **Quick-gDNA[™] MiniPrep** (D3024) and the **ZR Whole-Blood RNA MiniPrep** (R1020).

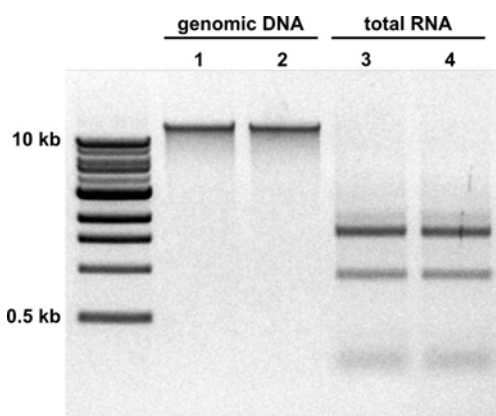
ZYMO RESEARCH CORP.

Toll Free: 1-888-882-9682 • Fax: 1-714-288-9643 • Web: www.zymoresearch.com • E-mail: info@zymoresearch.com

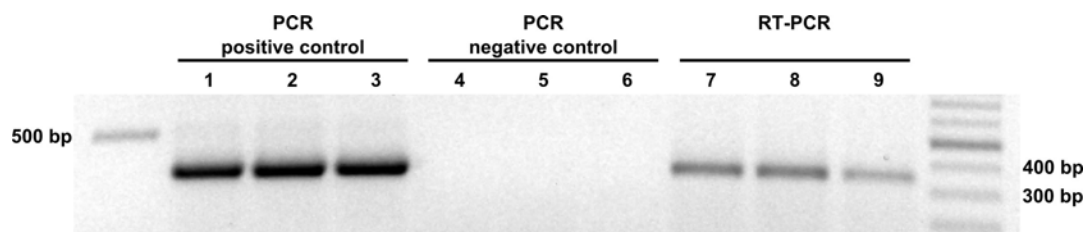
Product Description

The **ZR-Duet™ DNA/RNA MiniPrep** provides a quick method for the isolation of high quality genomic DNA and total RNA from small amounts of cells and tissue. The kit isolates *both* genomic DNA and a broad range of RNA species without the use of phenol. Small RNAs (e.g., tRNAs, microRNAs) can be recovered following a simple adjustment within the RNA isolation protocol – *no extra steps are required!* Both DNA and RNA from up to 5×10^6 cells can be eluted into volumes as little as 25 μ l in less than 15 minutes.

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



Genomic DNA (lane 1, 2) and total RNA (lane 3, 4) isolated from human epithelial cells (HCT 116) with the **ZR-Duet™ DNA/RNA MiniPrep**.



PCR amplification of β -actin transcript (353 bp fragment shown) following DNA and RNA isolation from human epithelial cells (HCT 116) with the **ZR-Duet™ DNA/RNA MiniPrep**: PCR positive control (DNA template; lane 1, 2, 3), PCR negative control (RNA template; lane 4, 5, 6), RT-PCR (lane 7, 8, 9).

Buffer Preparation

Before starting, add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate.

Protocol

1. **Sample Preparation**

- A. Adherent Cells:** Cells can be lysed directly in the culture container by removing liquid medium and adding **DNA/RNA Lysis Buffer**¹ directly to the monolayer (e.g., 400 μ l for 10^2 to 5×10^6 cells). Remove cells from the culture surface by pipetting, scraping, etc. Proceed to *Step 2*.
- B. Cells in Suspension:** Pellet the cells by gentle centrifugation (e.g., 5 minutes at 500 x g). Remove the supernatant completely and resuspend the cell pellet in 400 μ l **DNA/RNA Lysis Buffer**¹. Vortex briefly. Proceed to *Step 2*.
- C. Solid Tissue Samples:** Add 400 μ l **DNA/RNA Lysis Buffer**¹ to fresh or frozen tissue (up to ~25 mg) and homogenize the sample (e.g., using a Dounce or similar homogenizer). Proceed to *Step 2*.
- D. Liquid Samples:** Add 3 volumes of **DNA/RNA Lysis Buffer**¹ for every volume of sample (e.g., 300 μ l buffer to 100 μ l sample). Proceed to *Step 2*.

2. Transfer the sample from *Step 1* into a **Zymo-Spin™ IIC Column**^{2,3} in the **Collection Tube** and centrifuge at $\geq 12,000 \times g$ for 1 minute.

Save the flow-through for RNA and the column for DNA purification!

DNA Purification

3. Transfer the **Zymo-Spin™ IIC Column** into a new **Collection Tube**.
4. Add 200 μ l **DNA Prep Buffer** to the column and centrifuge at $\geq 12,000 \times g$ for 30 seconds.

RNA Purification⁴

3. Add 0.8 volume⁵ ethanol (95-100%) to the flow-through in the **Collection Tube**⁶ from *Step 2* (e.g., 320 μ l ethanol to 400 μ l flow-through) and mix well by pipetting.
4. Transfer the sample from *Step 3* into a **Zymo-Spin™ IIC Column**^{3,4} in a **Collection Tube** and centrifuge at $\geq 12,000 \times g$ for 1 minute. Discard the flow-through.⁷

Notes:

¹ In order to lyse samples completely, the amount of the **DNA/RNA Lysis Buffer** can be adjusted (i.e., more buffer can be added).

² The capacity of the **Zymo-Spin™ Column** is 800 μ l. Columns can be reloaded to process volumes >800 μ l.

³ The maximum binding capacity of the **Zymo-Spin™ IIC and IIC Column** is ~25 μ g of DNA/RNA.

⁴ Ensure the RNA isolation procedure is performed in an RNase-free environment.

⁵ For quantitative recovery of small RNAs (tRNAs, micro RNAs, etc.) use 2 volumes ethanol (95-100%).

⁶ Capacity of the **Collection Tube** is 2 ml.

⁷ **DNase I treatment:** Following *Step 4*, RNA samples can be DNase treated. See **Appendix** (page 5).

DNA Purification

5. Add 200 μ l **DNA Pre-Wash Buffer** to the column and centrifuge at $\geq 12,000 \times g$ for 1 minute. Discard the flow-through.
6. Add 500 μ l **g-DNA Wash Buffer** to the column and centrifuge at $\geq 12,000 \times g$ for 30 seconds. Discard the flow-through.
7. Centrifuge the **Zymo-Spin™ IIC Column** in an emptied **Collection Tube** at $\geq 12,000 \times g$ for 2 minutes. Remove the **Zymo-Spin™ IIC Column** carefully from the **Collection Tube** and transfer it into a clean microcentrifuge tube.
8. Add $\geq 50 \mu$ l **DNase/RNase-Free Water** directly to the column matrix and let stand 2 to 5 minutes at room temperature, then centrifuge at top speed for 30 seconds. The eluted DNA can be used immediately or stored at $\leq -20^\circ\text{C}$.

RNA Purification

5. Add 400 μ l **RNA Prep Buffer** to the column and centrifuge at $\geq 12,000 \times g$ for 1 minute. Discard the flow-through.
6. Add 700 μ l **RNA Wash Buffer** to the column and centrifuge at $\geq 12,000 \times g$ for 30 seconds. Discard the flow-through. Repeat the wash step with 400 μ l **RNA Wash Buffer**.
7. Centrifuge the **Zymo-Spin™ IIC Column** in an emptied **Collection Tube** at $\geq 12,000 \times g$ for 2 minutes. Remove the **Zymo-Spin™ IIC Column** carefully from the **Collection Tube** and transfer it into an RNase-free tube.
8. Add $\geq 25 \mu$ l **DNase/RNase-Free Water** directly to the column matrix and let stand 1 minute at room temperature. Centrifuge at $10,000 \times g$ for 30 seconds. The eluted RNA can be used immediately or stored at $\leq -70^\circ\text{C}$.

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.

Notes:

¹ 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 Catalog - **E1007**).

For *in-tube* DNase treatment and RNA clean-up see the **DNA-Free RNA Kit** (R1013) or the **RNA Clean & Concentrator™** (R1015, R1017).

To treat 1 μg RNA sample with DNase I, use of 1 unit enzyme is recommended.

Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A₂₆₀ units/min/ml of reaction mixture at 25°C.

² DNase I maintains activity in the **RNA Wash Buffer** provided in this kit.

Appendix

RNA purification with *in-column* DNase digestion¹

- Following *Step 1-4* in the Protocol, make 100 μl **DNase I** cocktail for each sample to be treated:

<i>Example:</i>	RNase-Free DNase I	10 μl (1 U/μl) ¹
	10x Reaction Buffer	10 μl
	RNA Wash Buffer ²	80 μl

- 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.
- Add 100 μl DNase I cocktail from *Step 1* directly to the matrix of the **Zymo-Spin™ IIC Column**. Keep the **Zymo-Spin™ IIC Column** in the **Collection Tube**.
- Incubate the column at 25-37°C for ≥15 minutes, then centrifuge at ≥12,000 x g for 30 seconds. Discard the flow-through.

Continue with *Step 6* in the standard Protocol.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR-Duet™ DNA/RNA MiniPrep	D7001	50 Preps.

For Individual Sale	Catalog No.	Amount
DNA/RNA Lysis Buffer	D7001-1-50	50 ml
DNA Prep Buffer	D7001-2-12	12 ml
DNA Pre-Wash Buffer	D3004-5-15	15 ml
	D3004-5-30	30 ml
	D3004-5-50	50 ml
g-DNA Wash Buffer	D3004-2-50	50 ml
	D3004-2-100	100 ml
RNA Prep Buffer	D3004-2-200	200 ml
	R1060-2-10	10 ml
RNA Wash Buffer (concentrate)	R1060-2-25	25 ml
	R1003-3-6	6 ml
	R1003-3-12	12 ml
	R1003-3-24	24 ml
DNase/RNase-Free Water	R1003-3-48	48 ml
	W1001-1	1 ml
	W1001-4	4 ml
	W1001-6	6 ml
Zymo-Spin™ IIC Columns	W1001-10	10 ml
	C1011-50	50
Zymo-Spin™ IIIC Columns	C1011-250	250
	C1006-50	50
Collection Tubes	C1006-250	250
	C1001-50	50
	C1001-500	500
	C1001-1000	1000

Related Products

Product	Description	Prep/Format	Catalog
Total RNA Purification			
ZR Whole-Blood RNA MiniPrep™	whole blood, partitioned blood	50/column 100/column	R1020 R1021
ZR-96 Whole-Blood RNA Kit™		2x96/plate	R1022
ZR Viral RNA Kit™	plasma, serum, liquids, cells, tissue	50/column 200/column	R1034 R1035
ZR-96 Viral RNA Kit™		2x96/plate 4x96/plate	R1040 R1041
ZR Urine RNA Isolation Kit™	urine, liquid samples	50/column	R1039
Quick-RNA™ MicroPrep	cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids	50/column	R1050
Quick-RNA™ MiniPrep		50/column 200/column	R1054 R1055
Quick-RNA™ MidiPrep		25/column	R1056
ZR-96 Quick-RNA™		2x96/plate 4x96/plate	R1052 R1053
ZR RNA MicroPrep™	cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids; DNA removal column, small-RNA recovery (≥17nt), <i>in-column</i> DNase treatment protocol	50/column 200/column	R1060 R1061
ZR RNA MiniPrep™		50/column 200/column	R1064 R1065
Pinpoint™ Slide RNA Isolation System Kit I	fresh tissue sections	50/column	R1003
Pinpoint™ Slide RNA Isolation System Kit II	paraffin-embedded tissue	50/column	R1007
ZR Fungal/Bacterial RNA MicroPrep™	bacteria, yeast, fungi; BashingBead™ lysis	50/column	R2010
ZR Fungal/Bacterial RNA MiniPrep™		50/column	R2014
ZR Plant RNA MiniPrep™	plant parts and tissues; BashingBead™ lysis, RT/PCR inhibitor removal	50/column	R2024
ZR Tissue & Insect RNA MicroPrep™	insect, small tissue samples; BashingBead™ lysis	50/column	R2030
YeaStar RNA Kit™	yeast, fungi	50/column	R1002
RNA Clean-up, Concentration & Recovery			
RNA Clean & Concentrator™-5	modified/labeled/impure/diluted RNA; small-RNA recovery (≥17nt); <i>acid phenol</i> extracted RNA directly from aqueous phase, <i>in-column</i> DNase treatment protocol	50/column 200/column	R1015 R1016
RNA Clean & Concentrator™-25		50/column 100/column	R1017 R1018
RNA Clean & Concentrator™-100		25/column	R1019
ZR-96 RNA Clean & Concentrator™		2x96/plate	R1080
DNA-Free RNA Kit™	DNase I treatment; small-RNA recovery (≥17nt)	50/column 200/column	R1013 R1014
Zymoclean™ Gel RNA Recovery Kit	agarose gel separated RNA	50/column	R1011
ZR small-RNA™ PAGE Recovery Kit	polyacrylamide gel separated RNA; small-RNA recovery (≥17nt)	20/column	R1070
DNA/RNA Parallel Purification			
ZR-Duet™ DNA/RNA MiniPrep	cells, tissue, liquids; DNA/RNA separation, small-RNA recovery (≥17nt), <i>in-column</i> DNase treatment protocol	50/column	D7001