



**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  $\geq 25 \mu\text{l}$  is suitable for use in PCR, RT-PCR, and other procedures.
- Omits the use of organic denaturants and proteases.

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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- <i>Duet</i> <sup>™</sup> 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 <sup>1</sup> (concentrate)               | 12 ml             | Room Temp.          |
| DNase/RNase-Free Water                                   | 10 ml             | Room Temp.          |
| Zymo-Spin <sup>™</sup> IIC Columns                       | 50                | Room Temp.          |
| Zymo-Spin <sup>™</sup> 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.*<sup>2</sup>
- **Sample Size** – 10<sup>2</sup> to 5x10<sup>6</sup> 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 - <sup>™</sup> 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<sup>late</sup><sup>™</sup> is a trademark of Ambion, Inc., Austin, Texas and is protected by various U.S. and foreign patents.

### Notes:

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

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

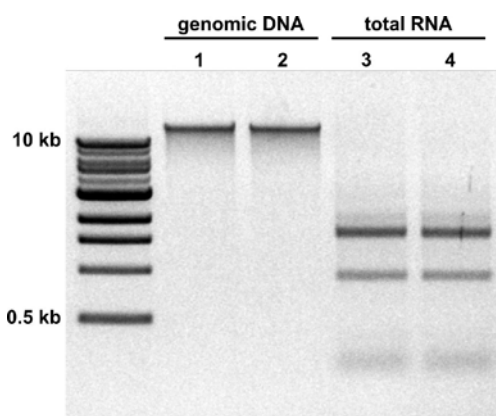
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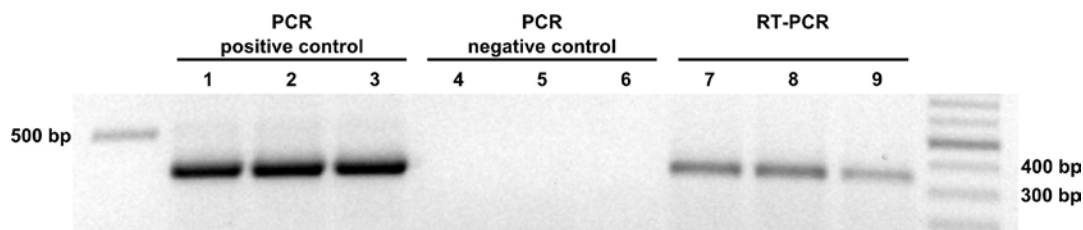
## Product Description

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  $5 \times 10^6$  cells can be eluted into volumes as little as 25  $\mu$ 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](mailto: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  $\beta$ -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**<sup>1</sup> directly to the monolayer (e.g., 400  $\mu$ l for  $10^2$  to  $5 \times 10^6$  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  $\mu$ l **DNA/RNA Lysis Buffer**<sup>1</sup>. Vortex briefly. Proceed to *Step 2*.
- C. Solid Tissue Samples:** Add 400  $\mu$ l **DNA/RNA Lysis Buffer**<sup>1</sup> 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**<sup>1</sup> for every volume of sample (e.g., 300  $\mu$ l buffer to 100  $\mu$ l sample). Proceed to *Step 2*.

2. Transfer the sample from *Step 1* into a **Zymo-Spin™ IIC Column**<sup>2,3</sup> 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  $\mu$ l **DNA Prep Buffer** to the column and centrifuge at  $\geq 12,000 \times g$  for 30 seconds.

### **RNA Purification**<sup>4</sup>

3. Add 0.8 volume<sup>5</sup> ethanol (95-100%) to the flow-through in the **Collection Tube**<sup>6</sup> from *Step 2* (e.g., 320  $\mu$ l ethanol to 400  $\mu$ l flow-through) and mix well by pipetting.
4. Transfer the sample from *Step 3* into a **Zymo-Spin™ IIC Column**<sup>3,4</sup> in a **Collection Tube** and centrifuge at  $\geq 12,000 \times g$  for 1 minute. Discard the flow-through.<sup>7</sup>

#### Notes:

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

<sup>2</sup> The capacity of the **Zymo-Spin™ Column** is 800  $\mu$ l. Columns can be reloaded to process volumes >800  $\mu$ l.

<sup>3</sup> The maximum binding capacity of the **Zymo-Spin™ IIC and IIC Column** is ~25  $\mu$ g of DNA/RNA.

<sup>4</sup> Ensure the RNA isolation procedure is performed in an RNase-free environment.

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

<sup>6</sup> Capacity of the **Collection Tube** is 2 ml.

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

### **DNA Purification**

5. Add 200  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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:

<sup>1</sup> 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 A<sub>260</sub> units/min/ml of reaction mixture at 25°C.*

<sup>2</sup> DNase I maintains activity in the **RNA Wash Buffer** provided in this kit.

## Appendix

### RNA purification with *in-column* DNase digestion<sup>1</sup>

- 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) <sup>1</sup> |
|                 | 10x Reaction Buffer                 | 10 μl                       |
|                 | <b>RNA Wash Buffer</b> <sup>2</sup> | 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 5* 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 |
|                               | D3004-2-200 | 200 ml |
| RNA Prep Buffer               | R1060-2-10  | 10 ml  |
|                               | R1060-2-25  | 25 ml  |
| RNA Wash Buffer (concentrate) | R1003-3-6   | 6 ml   |
|                               | R1003-3-12  | 12 ml  |
|                               | R1003-3-24  | 24 ml  |
|                               | R1003-3-48  | 48 ml  |
| DNase/RNase-Free Water        | W1001-1     | 1 ml   |
|                               | W1001-4     | 4 ml   |
|                               | W1001-6     | 6 ml   |
|                               | W1001-10    | 10 ml  |
| Zymo-Spin™ IIC Columns        | C1011-50    | 50     |
|                               | C1011-250   | 250    |
| Zymo-Spin™ IIIC Columns       | C1006-50    | 50     |
|                               | C1006-250   | 250    |
| Collection Tubes              | C1001-50    | 50     |
|                               | C1001-500   | 500    |
|                               | C1001-1000  | 1000   |

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## Related Products

| Product   | Description   | Prep/Format              | Catalog        |
|---|---|--------------------------|----------------|
| <b>Total RNA Purification</b>                     |   |                          |                |
| ZR Whole-Blood RNA MiniPrep™                      | whole blood, partitioned blood  | 50/column<br>100/column  | R1020<br>R1021 |
| ZR-96 Whole-Blood RNA Kit™                        |   | 2x96/plate               | R1022          |
| ZR Viral RNA Kit™                                 | plasma, serum, liquids, cells, tissue   | 50/column<br>200/column  | R1034<br>R1035 |
| ZR-96 Viral RNA Kit™                              |   | 2x96/plate<br>4x96/plate | R1040<br>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<br>200/column  | R1054<br>R1055 |
| Quick-RNA™ MidiPrep                               |   | 25/column                | R1056          |
| ZR-96 Quick-RNA™                                  |   | 2x96/plate<br>4x96/plate | R1052<br>R1053 |
| ZR RNA MicroPrep™                                 | cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids;<br>DNA removal column, small-RNA recovery (≥17nt), <i>in-column</i> DNase<br>treatment protocol | 50/column<br>200/column  | R1060<br>R1061 |
| ZR RNA MiniPrep™                                  |   | 50/column<br>200/column  | R1064<br>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          |
| <b>RNA Clean-up, Concentration &amp; Recovery</b> |   |                          |                |
| 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<br>treatment protocol | 50/column<br>200/column  | R1015<br>R1016 |
| RNA Clean & Concentrator™-25                      |   | 50/column                | R1017          |
| RNA Clean & Concentrator™-100                     |   | 100/column               | R1018          |
| ZR-96 RNA Clean & Concentrator™                   |   | 25/column                | R1019          |
| DNA-Free RNA Kit™                                 | DNase I treatment; small-RNA recovery (≥17nt)   | 2x96/plate               | R1080          |
| Zymoclean™ Gel RNA Recovery Kit                   |   | 50/column<br>200/column  | R1013<br>R1014 |
| ZR small-RNA™ PAGE Recovery Kit                   | agarose gel separated RNA   | 50/column                | R1011          |
|   | polyacrylamide gel separated RNA; small-RNA recovery (≥17nt)  | 20/column                | R1070          |
| <b>DNA/RNA Parallel Purification</b>              |   |                          |                |
| ZR-Duef™ DNA/RNA MiniPrep                         | cells, tissue, liquids; DNA/RNA separation, small-RNA recovery (≥17nt),<br><i>in-column</i> DNase treatment protocol  | 50/column                | D7001          |

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