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 (~25 µg) from a wide range of sources using Clean-Spin™ column technology.
- High-quality DNA/RNA eluted in ≥25 µl is ready for reverse transcription, microarray, sequencing, etc.

Contents

ZYMO RESEARCH CORP.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

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-*Duet*™ DNA/RNA MiniPrep (Kit Size)	**D7001** (50 preps.)
DNA/RNA Lysis Buffer	50 ml
DNA/RNA Prep Buffer	50 ml
DNA/RNA Wash Buffer[1] (concentrate)	24 ml
DNase/RNase-Free Water	10 ml
Zymo-Spin™ IIC Columns	50
Zymo-Spin™ IIIC Columns	50
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.

Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.

[1] Ethanol must be added prior to use as indicated on **DNA/RNA Wash Buffer** label.

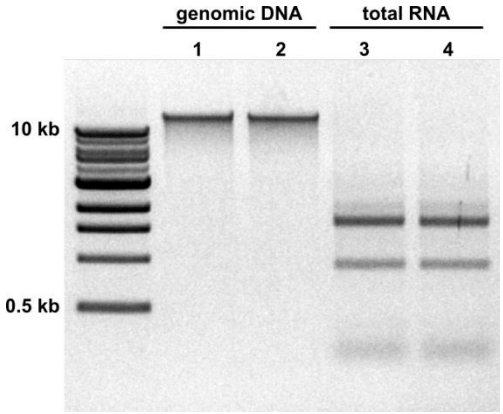
Specifications

- **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** – 10^2 to $5x10^6$ 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 (page 5).
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge

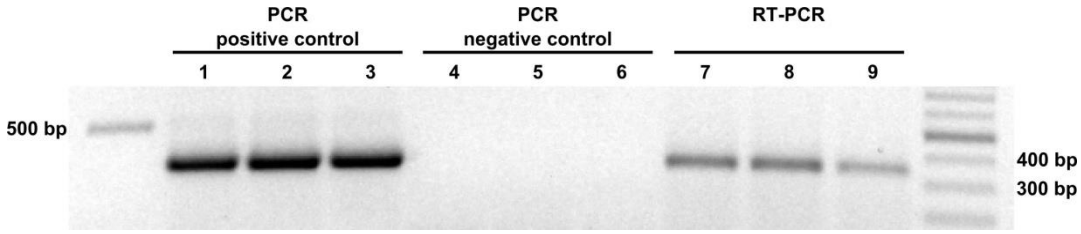
[2] For purification of DNA and RNA from whole blood, see the **ZR-*Duet*™ DNA/RNA MiniPrep Plus** (D7003).

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 $5x10^6$ cells can be eluted into volumes as little as 25 µl in less than 15 minutes.



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).

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

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

Buffer Preparation

Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/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 µl for 10^2 to $5x10^6$ 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 µl **DNA/RNA Lysis Buffer**[1]. Vortex briefly. Proceed to *Step 2*.

   C. Solid Tissue Samples: Add 400 µl **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 µl buffer to 100 µl sample)*. Proceed to *Step 2*.

2. Transfer the sample from *Step 1* into a **Zymo-Spin™ IIIC Column**[2,3] in the **Collection Tube** and centrifuge at ≥12,000 x *g* for 1 minute.

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

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 µl. Columns can be reloaded to process volumes >800 µl.

[3] The maximum binding capacity of the **Zymo-Spin™ IIIC and IIC Column** is ~25 µg of DNA/RNA.

DNA Purification	*RNA Purification*
3. Transfer the **Zymo-Spin™ IIIC Column** into a new **Collection Tube**.	3. Add 1 volume[1] ethanol (95-100%) to the flow-through in the **Collection Tube**[2] from *Step 2* and mix well by pipetting. Then transfer the sample into a **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge at ≥12,000 x *g* for 1 minute. Discard the flow-through.[3]

4. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge at ≥12,000 x *g* for 1 minute. Discard the flow-through.

5. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge at ≥12,000 x *g* for 30 seconds. Discard the flow-through.

6. Add 400 µl **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into a clean microcentrifuge tube.

7. Add ≥50 µl **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 ≤-20ºC.	7. Add ≥25 µl **DNase/RNase-Free Water** directly to the column matrix and let stand 1 minute at room temperature. Centrifuge at 10,000 x *g* for 30 seconds. The eluted RNA can be used immediately or stored at ≤-70°C.

Notes:

[1] Alternatively, to isolate RNAs ≥200 nt, add ½ volume ethanol (95-100%) to the sample flow-through.

[2] Capacity of the **Collection Tube** is 2 ml.

[3] At this point, RNA samples can be in-column DNase I treated (page 5).

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

** Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.*

In-Column **DNase I Digestion**

The DNase I digestion procedure can be performed using **DNase I Set** (E1010).[1]
All centrifugation steps should be performed at 10,000 –16,000 *x g* for 30 seconds unless specified.

1. Wash the column with 400 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.

2. Add 80 µl **DNase I Reaction Mix** (below) directly to the column matrix.

DNase I	5 µl (1 U/µl)*
DNA Digestion Buffer	75 µl

3. Incubate the column at room temperature (20-30ºC) for 15 minutes.
Continue with RNA Purification: Page 4, Step 4.

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/RNA Prep Buffer	D7010-2-10 D7010-2-25 D7010-2-50	10 ml 25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-6 D7010-3-12 D7010-3-24	6 ml 12 ml 24 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
Zymo-Spin™ IIC Columns	C1011-50 C1011-250	50 250
Zymo-Spin™ IIIC Columns	C1006-50 C1006-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 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), *in-column* 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); *acid phenol* extracted RNA directly from aqueous phase, *in-column* 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), *in-column* DNase treatment protocol	50/column	**D7001**