ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-*Duet*™ DNA/RNA MiniPrep Plus

Catalog No. **D7003**

Highlights

- Efficient isolation and separation of DNA and RNA from any cells, tissue, blood, and biological fluids.
- High quality DNA and *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

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.

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

Product Contents

ZR-*Duet*™ DNA/RNA MiniPrep Plus (Kit Size)	**D7003** (50 Preps.)	**Storage Temperature**
DNA/RNA Lysis Buffer	50 ml	Room Temp.
DNA/RNA Prep Buffer	50 ml	Room Temp.
DNA/RNA Wash Buffer[1] (concentrate)	24 ml	Room Temp.
DNase/RNase-Free Water	10 ml	Room Temp.
DNase I[2] (lyophilized)	1	-20ºC (reconstituted)
DNA Digestion Buffer	4 ml	Room Temp.
DNA/RNA Shield™ (2X concentrate)	25 ml	Room Temp.
PK Digestion Buffer	5 ml	Room Temp.
Proteinase K[3] (w/ Storage Buffer)	20 mg	-20ºC (reconstituted)
Spin-Away™ **Filters**	50	Room Temp.
Zymo-Spin™ **IIICG Columns**	50	Room Temp.
Collection Tubes	3x 50	Room Temp.
Instruction Manual	1	-

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

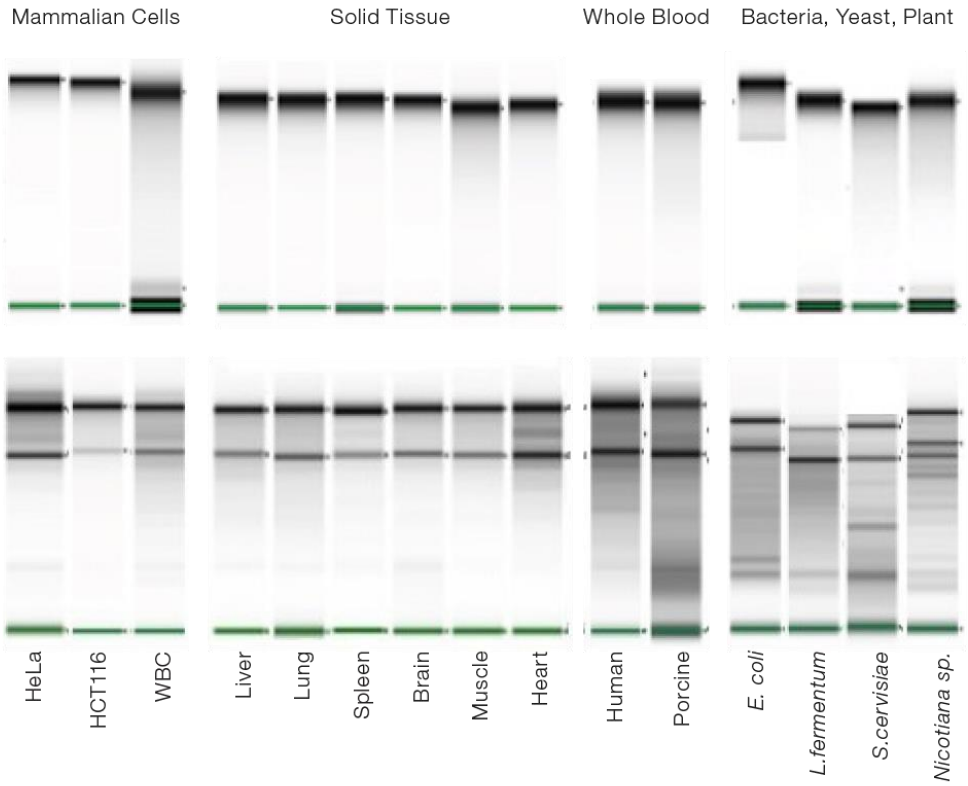
[1] Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate before use.
[2] Prior to use, reconstitute the lyophilized DNase I with 275 µl DNase/RNase-Free Water. Mix by inversion. Store aliquots at -20ºC.
[3] Prior to use, reconstitute the lyophilized **Proteinase K** with 1040 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20ºC.

Specifications

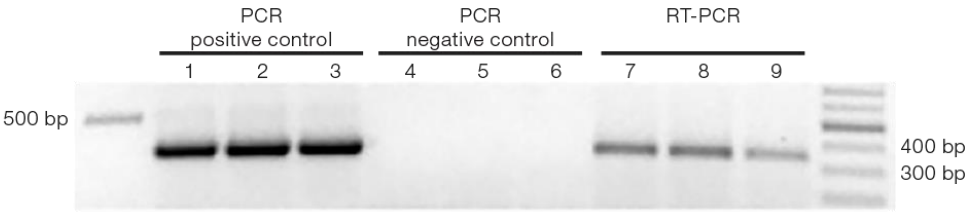
- **Sample Sources** – Any cells (animal, blood cells, *etc.*), all tissues (tough-to-lyse, FFPE, *etc.*), blood, biological fluids, and samples in DNA/RNA Shield™.
- **Sample Preservation** – **DNA/RNA Shield**™ lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures (page 7).
- **Size Limits** – Capable of recovering genomic DNA ≥40 kb and total RNA ≥17 nt.
- **Purity** – High quality genomic DNA and RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) are recovered.
- **Recovery** – The DNA/RNA binding capacity of the included columns is 100 µg.
- **Storage** – DNA and RNA is eluted with DNase/RNase-free water and can be stored frozen. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, and 55°C heat block, water bath or incubator.

Product Description

The **ZR-*Duet*™ DNA/RNA MiniPrep Plus** kit combines **ZR-*Duet*™** technology with the addition of **DNA/RNA Shield**™, a unique preservation and lysis technology, and **Proteinase K** to enable easy, reliable, and rapid isolation from any biological sample including cells, solid tissue, and whole blood. The procedure uses *Zymo-Spin*™ column technology that results in high-quality genomic DNA and total RNA that is ready for any downstream application including reverse transcription, microarray, sequencing, *etc*.



High quality genomic DNA (top) and total RNA (bottom) are isolated from various sample types including mammalian cells, solid tissue, whole blood, bacteria, yeast, and plant using the **ZR-*Duet*™ DNA/RNA MiniPrep Plus** kit (Agilent 2200 TapeStation).



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 Plus**: 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.

Purification Guide

Sample Input	Start on
Cells (mammalian, blood cells)	**Page 4**
Tissue (soft, fibrous, lipid, FFPE)	
Whole Blood (biological liquids)	**Page 5**
Tough-to-Lyse (bacteria, yeast, plant)	
Preserved Samples (DNA/RNA Shield™, RNAlater™)	

DNA & RNA Yields and Kit Capacity

Sample Input	Average gDNA Yield	Average RNA Yield	Kit Capacity
Cells	4 µg (per 10^6 cells)	10 µg (per 10^6 cells)	Up to 10^7
HeLa	6 µg	15 µg	
High Yield Tissue[1] (mouse)	≥30 µg (per 10 mg)	≥30 µg (per 10 mg)	Up to 20 mg
Spleen	50-70 µg	30-50 µg	
Liver	15-30 µg	40-60 µg	
Low Yield Tissue[1] (mouse)	≤30 µg (per 10 mg)	≤30 µg (per 10 mg)	Up to 50 mg
Brain, Heart	5-15 µg	5-15 µg	
Muscle	5-15 µg	5-20 µg	
Lung	15-30 µg	10-20 µg	
Intestine	15-30 µg	10-30 µg	
Kidney	15-30 µg	20-30 µg	
Whole Blood[2]	(per 1 ml)	(per 1 ml)	Up to 1 ml
Porcine	5-10 µg	10-20 µg	
Human	2-5 µg	2-10 µg	

Notes:

[1] Yield from tissue samples can vary due to other factors such as organism type, physiological state, and growth conditions.

[2] Yield from blood samples can vary based upon the donor, age, and/or health conditions.

Reagent Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
- ✓ Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** at 1 U/µl. Mix by gentle inversion. Store frozen aliquots at -20ºC.
- ✓ Add 1040 µl **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store at -20ºC.

The lyophilized **Proteinase K** and **DNase I** are stable as shipped.

Protocols

The isolation consists of two steps: (I) Sample Preparation & (II) DNA/RNA Purification.

Sample Preparation

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.

Cells

Pellet[1] mammalian cells by centrifugation (≤500 *x g* for 1 minute), remove the supernatant and resuspend the cell pellet in **DNA/RNA Lysis Buffer**[2] (see table below). Proceed to Page 6.

Mammalian Cells	Add DNA/RNA Lysis Buffer
≤5x10^6	300 µl
5x10^6 - 10^7	≥600 µl

Solid Tissue & Blood Cells (PBMCs, WBCs)

1. Add **DNA/RNA Shield**™ (1X)[3] to a solid tissue sample (see table below).
   Tissue samples can be mechanically homogenized for optimal extraction efficiency.
   For blood cells, buffy coat and pelleted PAXgene™ samples, resuspend in **DNA/RNA Shield**™ (1X).

Animal Tissue	Blood Cells	Add DNA/RNA Shield™ (1X)
≤50 mg	≤5 ml blood	≥300 µl[4]

2. For every 300 µl of sample, add 30 µl **PK Digestion Buffer** and 15 µl **Proteinase K**.
3. Mix and then incubate at 55ºC until tissue dissolves or up to 5 hours.[5]

Sample	Incubation Time
Non-homogenized	2-5 hours
Homogenized tissue	30 minutes
Blood cells (or PAXgene™ pellet)	30 minutes

4. After incubation, vortex sample and then centrifuge at max speed for 2 minutes to pellet debris. Transfer the aqueous supernatant into an RNase-free tube (not provided).
5. Add an equal volume of **DNA/RNA Lysis Buffer** and mix well. Proceed to Page 6.

Notes:

[1] Cells in suspension and other liquids may be processed directly by adding 4 volumes of **DNA/RNA Lysis Buffer** and mixing. Proceed to Page 6.

[2] Cell samples homogenized in **DNA/RNA Lysis Buffer** can be stored frozen for processing at a later time.

[3] To prepare 1X solution, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided).

FFPE tissue (page 7).

[4] Solid tissue samples should be completely submerged in **DNA/RNA Shield**™, add as needed.

[5] Optimal incubation times may vary with tissue type and homogenization method.

Notes:

[1] Up to 200 µl can be processed without having to reload the spin column.

With reloading, up to 1 ml blood per prep can be processed.

**For bacterial, fungal, fecal, and soil samples, use the 0.5 mm beads (S6002).*

**For plant/seed, solid tissues, and insect samples, use the 2.0 mm beads (S6003).*

[2] Some Gram-negative bacteria (*e.g. E. coli*) may not require the **BashingBead™** system and can be lysed directly in **DNA/RNA Shield™**.

Sample preservation with DNA/RNA Shield™ (page 7).

Sample Preparation (continued)

Whole Blood (Mammalian)

1. Add 200 µl[1] **DNA/RNA Shield™** (2X concentrate) directly to each 200 µl of fresh/frozen blood sample and mix thoroughly.
2. For every 400 µl of reagent/blood mixture, add 8 µl **Proteinase K** and mix thoroughly. Incubate at room temperature (20-30ºC) for 30 minutes.
3. Add an equal volume of isopropanol and mix by vortex. Proceed to Page 6.

Tough-to-Lyse Samples (Bacterial, Yeast, Plant, *etc.*)

Tough-to-lyse samples (including gram-positive bacteria) should be mechanically homogenized (*i.e.* ZR BashingBead™ Lysis Tubes*) directly in **DNA/RNA Shield™** (1X; mix equal amounts of the supplied 2X concentrate with nuclease-free water (not supplied)). Centrifuge and transfer the supernatant into an RNase-free tube. Add an equal volume of **DNA/RNA Lysis Buffer** and mix well. Proceed to Page 6.

Bacterial[2]	Yeast	Plant/Seed	Add DNA/RNA Shield™ (1X)
≤5x10^7	≤5x10^6	-	400 µl
5x10^7 - 10^9	5x10^6 - 5x10^7	≤200 mg	≥800 µl

For the removal of PCR (RT) inhibitors from fecal, soil, plant, use the OneStep™ PCR Inhibitor Removal Kit (D6030).

Liquids

Add 3 volumes of **DNA/RNA Lysis Buffer** for every volume of sample (*e.g.* 300 µl buffer to 100 µl sample). Proceed to Page 6.

Samples Preserved in DNA/RNA Shield™

Bring samples homogenized and stored in **DNA/RNA Shield™** (1X) to room temperature (20-30ºC). Add 1 volume of **DNA/RNA Lysis Buffer** (1:1) and mix well. Proceed to Page 6.

Samples in RNA*later*™

To process cells or liquids in RNA*later*™ (without reagent removal): Add 1 volume of RNase-free water or PBS to the sample (1:1). Then add 4 volumes **DNA/RNA Lysis Buffer** (4:1) and mix. Proceed to Page 6.

Note: Alternatively, remove the RNA*later*™, then proceed with Sample Preparation according to the sample.

Purification Protocol

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.

1. Transfer the sample into a **Spin-Away™ Filter**[1] (**yellow**) in a **Collection Tube** and centrifuge.[2]

   Save the flow-through.

   For whole blood only:

   Discard the flow-through from above. Transfer the filter into a clean microcentrifuge tube (not provided). Add 200 µl **DNA/RNA Lysis Buffer** directly to the filter matrix, let stand 5 minutes and then centrifuge. **Save the flow-through.**

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

DNA Purification (DNA is bound to the column)	RNA Purification (RNA is in the flow-through)
2. Transfer the **Spin-Away™ Filter** (**yellow**) into a new **Collection Tube**.	2. Add 1 volume[3] ethanol (95-100%) to the flow-through and mix well. Then transfer the sample into a **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge. Discard the flow-through.[4,5]

3. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
4. Add 700 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.
5. 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.
6. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix, let stand for 5 minutes, and then centrifuge to elute DNA and RNA from the respective column.

   Alternatively, for highly concentrated DNA and RNA, use ≥50 µl elution.

   The eluted DNA & RNA can be used immediately or stored at ≤-70ºC.

Notes:

[1] To process samples >700 µl, **Zymo-Spin™** columns may be reloaded.

[2] Whole blood: For processing large volumes, a vacuum manifold can be used for this step. Following the vacuum step, centrifuge the column to remove residual liquid.

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

[4] Save the flow-through for protein purification (page 7).

[5] At this point, RNA samples can be in-column DNase I treated (page 7).

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.*

Appendices

Sample Preservation in DNA/RNA Shield™

DNA/RNA Shield™ effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume DNA/RNA Shield™ (2X concentrate) and sample.
Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in DNA/RNA Shield (1X).

Mix well/homogenize sample prior to storage.

Samples in DNA/RNA Shield™ can be stored at ambient temperature (4-25ºC) for a month, 3 days at 37ºC or long term (>1 year) at -20ºC or below.

In-Column DNase I Treatment

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 6, Step 3.

Acetone Precipitation of Proteins

1. Add 4 volumes of cold acetone (-20 ºC) to flow-through with ethanol obtained after RNA binding in *RNA Purification.*
2. Incubate samples for 30 minutes on ice.
3. Centrifuge at top speed for 10 minutes. Discard the supernatant. Keep the pellet!
4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at top speed for 1 minute. Discard the supernatant.
5. Air-dry protein pellet for 10 minutes at room temperature.
6. Resuspend and vortex the pellet in a buffer appropriate for downstream application. For example: SDS-PAGE sample loading buffer.

DNA/RNA Purification from FFPE Tissue: Deparaffinization

1. Remove (trim) as much excess paraffin from the sample as possible.
2. Transfer sample to a microcentrifuge tube (not provided).
3. Add 1 ml xylene and mix well. Then centrifuge for 1 minute and remove xylene.
4. Add 1 ml ethanol (95-100%) and mix well. Then centrifuge for 1 minute and remove ethanol. Repeat this step.
5. Dry samples by vacuum centrifugation (Speed-Vac) or by incubating uncapped tubes at ≤37 °C. It may take up to 40 minutes for a sample to air dry.
6. To purify DNA/RNA, follow Sample Preparation for tissue (Page 4) and DNA/RNA Purification (Page 6).

Troubleshooting:

For **Technical Assistance**, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com.

Problem	Possible Causes and Suggested Solutions
Sample Degradation	Sample Preservation • Immediately submerge tissue samples in **DNA/RNA Shield™** to ensure nucleic acid stability. • Using frozen whole blood (EDTA, citrate) samples can cause cell damage from membrane shock and shearing, resulting in degradation. For best results, store fresh whole blood in DNA/RNA Shield™ (2X concentrate). Alternatively, add the DNA/RNA Shield to frozen blood samples prior to thawing.
Low Yield	Sample Input • For "high yield" samples, if the lysate is extremely viscous or did not pass through the column, use less input material. Too much input can cause cellular debris to overload the column and result in compromised DNA/RNA recovery. • For "low yield" tissue (e.g., muscle), using larger inputs will increase yields (≤ 50 mg). Refer to the "DNA & RNA Yields" table (page 3). Incomplete Lysis/Digestion • Proteinase K incubation times may be extended depending on the type of sample (e.g., fibrous tissues). Elution • Reload the eluate onto the column and centrifuge again. Alternatively, heat the nuclease-free water to 95ºC before use.
Residual DNA	DNA Removal • Perform *in-column* DNase I treatment (page 7) to remove DNA from the RNA fraction. For "high-yield" samples, do not overload the Spin-Away™ Filter (titrate the input if necessary).
Low $A_{260/230\ nm}$	Sample Handling • There may be ethanol and/or salt contamination. Carefully remove the column from the collection tube so that there is no liquid contact. Blot emptied collection tubes with a tissue or towel to minimize liquid retention. • Make sure lysate has passed completely through matrix in the column before proceeding to wash steps. This may require centrifuging at a higher speed and/or longer time.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR-*Duet*™ DNA/RNA MiniPrep	D7001	50 Preps.
ZR-*Duet*™ DNA/RNA MiniPrep Plus	D7003	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
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
PK Digestion Buffer	R1200-1-5 R1200-1-20	5 ml 20 ml
Proteinase K (lyophilized) supplied with Proteinase K Storage Buffer	D3001-2-5 D3001-2-20	5 mg set 20 mg set
Spin-Away™ Filters	C1006-50-F C1006-250-F	50 250
Zymo-Spin™ IIICG Columns	C1006-50-G C1006-250-G	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

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