



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## **Quick-DNA/RNA™ Microprep Plus Kit**

Catalog No. **D7005**

### **Highlights**

- Efficient isolation and separation of DNA and RNA from the same small sample of a wide range of sources including cells, tissue (biopsy), blood, and biological fluids.
- High quality DNA and *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

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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](mailto:tech@zymoresearch.com).

## Product Contents

<b>Quick-DNA/RNA™ Microprep Plus Kit (Kit Size)</b>	<b>D7005 (50 Preps.)</b>	<b>Storage Temperature</b>
<b>DNA/RNA Lysis Buffer</b>	50 ml	Room Temp.
<b>DNA/RNA Prep Buffer</b>	50 ml	Room Temp.
<b>DNA/RNA Wash Buffer<sup>1</sup> (concentrate)</b>	2x 24 ml	Room Temp.
<b>DNase/RNase-Free Water</b>	10 ml	Room Temp.
<b>DNase I<sup>2</sup> (lyophilized)</b>	1	-20°C (reconstituted)
<b>DNA Digestion Buffer</b>	4 ml	Room Temp.
<b>DNA/RNA Shield™ (2X concentrate)</b>	25 ml	Room Temp.
<b>PK Digestion Buffer</b>	5 ml	Room Temp.
<b>Proteinase K<sup>3</sup> (w/ Storage Buffer)</b>	20 mg	-20°C (reconstituted)
<b>Zymo-Spin™ IC-XM Columns</b>	50	Room Temp.
<b>Zymo-Spin™ IC Columns</b>	50	Room Temp.
<b>Collection Tubes</b>	3x 50	Room Temp.
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**Storage Temperature** - Store all kit components (*i.e.*, buffers, columns) at room temperature.

<sup>1</sup> Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate before use.

<sup>2</sup> Prior to use, reconstitute the lyophilized DNase I with 275 µl DNase/RNase-Free Water. Mix by inversion. Store aliquots at -20°C.

<sup>3</sup> Prior to use, reconstitute the lyophilized **Proteinase K** with 1040 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20°C.

## Specifications

- **Sample Sources** – Any cells (animal, blood cells, *etc.*), tissue biopsies (tough-to-lyse, FFPE, *etc.*), microdissections (LCM), needle aspirates, 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** – Maximum binding capacity of the provided columns is 5 µg for DNA and 10 µg for RNA.
- **Storage** – DNA and RNA is eluted with DNase/RNase-free water and can be stored at ≤-70 °C. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, and 55°C heat block, water bath or incubator.

™ Trademarks of Zymo Research Corporation. This product is for research use only and should 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 safety guidelines and rules enacted by your research institution or facility. RNAlater™ is a trademark of Ambion, Inc. PAXgene™ is a trademark of PreAnalytiX, GmbH.

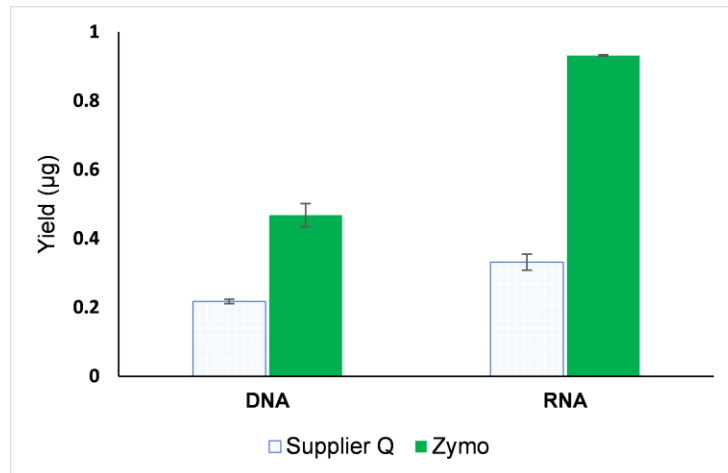
### ZYMO RESEARCH CORP.

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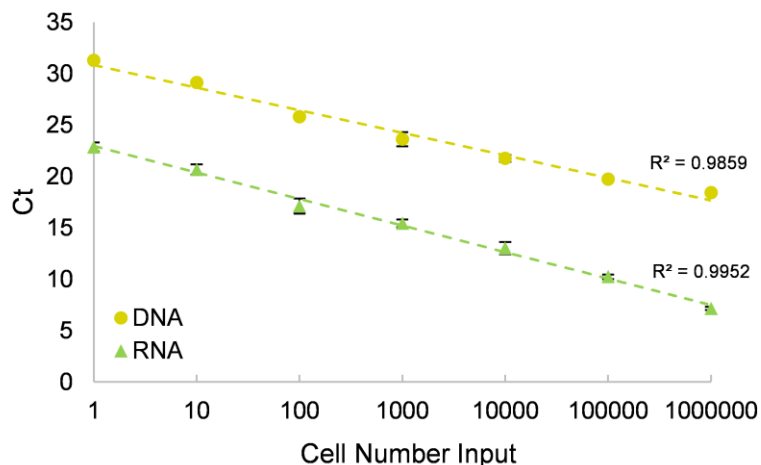
## Product Description

The **Quick-DNA/RNA™ MicroPrep Plus** kit combines **Quick-DNA/RNA™** 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 gDNA and total RNA that is ready for any downstream application including reverse transcription, microarray, sequencing, *etc.*

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



DNA and total RNA recovery is higher using the **Quick-DNA/RNA™ Microprep Plus Kit** compared to Supplier Q. Nucleic acids were extracted from 50K HeLa cells (n=2).



DNA and total RNA were extracted from a decreasing amount of HeLa cells using the **Quick-DNA/RNA™ Microprep Plus Kit**. Analysis by RT-qPCR shows high linear recovery of DNA & RNA down to the single cell level (n=2).

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## Purification Guide

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

## DNA & RNA Yields and Kit Capacity

Sample Input	Average gDNA Yield	Average RNA Yield	Kit Capacity
<b>Cells</b>	0.4 µg (per 10 <sup>5</sup> cells)	1 µg (per 10 <sup>5</sup> cells)	Up to 10 <sup>6</sup>
HeLa	0.6 µg	1.5 µg	
<b>High Yield Tissue</b> <sup>1 (mouse)</sup>	≥3 µg (per 1 mg)	≥3 µg (per 1 mg)	Up to 2 mg
Spleen	5-7 µg	3-5 µg	
Liver	1.5-3 µg	4-6 µg	
<b>Low Yield Tissue</b> <sup>1 (mouse)</sup>	≤3 µg (per 1 mg)	≤3 µg (per 1 mg)	Up to 5 mg
Brain, Heart	0.5-1.5 µg	0.5-1.5 µg	
Muscle	0.5-1.5 µg	0.5-2 µg	
Lung	1.5-3 µg	1-2 µg	
Intestine	1.5-3 µg	1-3 µg	
Kidney	1.5-3 µg	2-3 µg	
<b>Whole Blood</b> <sup>2</sup>	(per 100 µl)	(per 100 µl)	Up to 200 µl
Porcine	0.5-1 µg	1-2 µg	
Human	0.2-0.5 µg	0.2-1 µg	

### Notes:

<sup>1</sup> Yield from tissue samples can vary due to other factors such as organism type, physiological state, and growth conditions.

<sup>2</sup> 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  $\mu$ l **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** at 1 U/ $\mu$ l. Mix by gentle inversion. Store frozen aliquots at -20°C.
- ✓ Add 1040  $\mu$ l **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store at -20°C.

## 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 (20-30°C) unless specified.

### Cells

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

Mammalian Cells	Add DNA/RNA Lysis Buffer
$\leq 10^6$	400 $\mu$ l

### Solid Tissue & Blood Cells (PBMCs, WBCs)

1. Add **DNA/RNA Shield™** (1X)<sup>3</sup> 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)
$\leq 5$ mg	$\leq 0.5$ ml blood	$\geq 300$ $\mu$ l <sup>4</sup>

2. For every 300  $\mu$ l of sample, add 30  $\mu$ l **PK Digestion Buffer** and 15  $\mu$ l **Proteinase K**<sup>5</sup>.
3. Mix and then incubate at 55°C until tissue dissolves or up to 5 hours.<sup>6</sup>

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.

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

#### Notes:

<sup>1</sup> 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.

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

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

FFPE tissue (page 7).

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

<sup>5</sup> For protein purification, omit step 2 and continue to step 5.

<sup>6</sup> Optimal incubation times may vary with tissue type and homogenization method.

**Notes:**

<sup>1</sup> Up to 200 µl can be processed without having to reload the spin column.

<sup>2</sup> For protein purification, omit step 2 and continue to step 3.

*\*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).*

<sup>3</sup> 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<sup>1</sup> **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<sup>2</sup>** and mix thoroughly. Incubate at room temperature (20-30°C) 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 <sup>3</sup>	Yeast	Plant/Seed	Add DNA/RNA Shield™ (1X)
≤2x10 <sup>8</sup>	≤2x10 <sup>7</sup>	≤20 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 (20-30°C). Add 1 volume of **DNA/RNA Lysis Buffer** (1:1) and mix well. Proceed to Page 6.

### **Samples in RNA/ater™**

To process cells or liquids in RNA/ater™ (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/ater™, 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 **Zymo-Spin™ IC-XM Column** in a **Collection Tube** and centrifuge.

**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)

2. Transfer the **Zymo-Spin™ IC-XM Column** into a new **Collection Tube**.

### **RNA Purification**

(RNA is in the flow-through)

2. Add 1 volume<sup>3</sup> ethanol (95-100%) to the flow-through and mix well. Then transfer the sample into a **Zymo-Spin™ IC Column**<sup>1</sup> in a **Collection Tube** and centrifuge. Discard the flow-through.<sup>4,5</sup>

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 15 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 ≥6 µl elution.

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

### **Notes:**

<sup>1</sup> To process samples >700 µl, **Zymo-Spin™** columns may be reloaded.

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

<sup>4</sup> Save the flow-through for protein purification (page 7).

<sup>5</sup> At this point, RNA samples can be in-column DNase I treated (page 7).

## 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 (4-25°C) for a month, 3 days at 37°C or long term (>1 year) at -20°C or below.

### In-Column DNase I Treatment

The DNase I digestion procedure can be performed using **DNase I Set** (E1010).<sup>1</sup>

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 40 µl **DNase I Reaction Mix** (below) directly to the column matrix.

<b>DNase I</b>	5 µl (1 U/µl)*
<b>DNA Digestion Buffer</b>	35 µl
3. Incubate the column at room temperature (20-30°C) for 15 minutes. Continue with RNA Purification: Page 6, Step 3.

### Acetone Precipitation of Proteins

1. Add 4 volumes of cold acetone (-20 °C) 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).

#### Notes:

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



**Ordering Information**

Product Description	Catalog No.	Kit Size
<b>Quick-DNA/RNA™ Microprep Plus Kit</b>	D7005	50 Preps.
<b>Quick-DNA/RNA™ Miniprep Kit</b>	D7001	50 Preps.
<b>Quick-DNA/RNA™ Miniprep Plus Kit</b>	D7003	50 Preps.

For Individual Sale	Catalog No.	Amount
<b>DNA/RNA Lysis Buffer</b>	D7001-1-50	50 ml
<b>DNA/RNA Prep Buffer</b>	D7010-2-10	10 ml
	D7010-2-25	25 ml
	D7010-2-50	50 ml
<b>DNA/RNA Wash Buffer</b> (concentrate)	D7010-3-6	6 ml
	D7010-3-12	12 ml
	D7010-3-24	24 ml
<b>DNase/RNase-Free Water</b>	W1001-1	1 ml
	W1001-4	4 ml
	W1001-6	6 ml
	W1001-10	10 ml
<b>DNase I Set</b> (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
<b>DNA/RNA Shield™</b> (2X concentrate)	R1200-25	25 ml
	R1200-125	125 ml
<b>PK Digestion Buffer</b>	R1200-1-5	5 ml
	R1200-1-20	20 ml
<b>Proteinase K</b> (lyophilized) supplied with Proteinase K Storage Buffer	D3001-2-5	5 mg set
	D3001-2-20	20 mg set
<b>Zymo-Spin™ IC-XM Columns</b>	C1103-50	50
<b>Zymo-Spin™ IC Columns</b>	C1004-50	50
	C1004-250	250
	C1001-50	50
<b>Collection Tubes</b>	C1001-500	500
	C1001-1000	1000

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