



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

**DNA  
Purification**  
XXXXXXXX *Made Simple™*

# INSTRUCTION MANUAL

## **Quick-DNA™ Miniprep Plus Kit**

Catalog Nos. **D4068T, D4068 & D4069**

### **Highlights**

- Extract high-quality DNA easily and reliably from any biological fluids, cultured/monolayer cells, or solid tissues.
- **Zymo-Spin™ Technology** ensures DNA is ready for all sensitive downstream applications such as qPCR, DNA-sequencing, arrays, and methylation analysis.

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## Product Contents

<b>Quick-DNA™ Miniprep Plus Kit (Kit Size)</b>	<b>D4068T (10 Preps.)</b>	<b>D4068 (50 Preps.)</b>	<b>D4069 (200 Preps.)</b>	<b>Storage Temperature</b>
<b>Proteinase K &amp; Storage Buffer</b>	5 mg	20 mg	4 x 20 mg	-20°C (after mixing)
<b>BioFluid &amp; Cell Buffer (Red)</b>	2.5 ml	12 ml	45 ml	Room Temp.
<b>Solid Tissue Buffer (Blue)*</b>	1.5 ml	6 ml	22 ml	Room Temp.
<b>Genomic Binding Buffer</b>	5 ml	25 ml	85 ml	Room Temp.
<b>DNA Pre-Wash Buffer</b>	5 ml	30 ml	2 x 50 ml	Room Temp.
<b>g-DNA Wash Buffer</b>	10 ml	50 ml	200 ml	Room Temp.
<b>DNA Elution Buffer</b>	1 ml	10 ml	50 ml	Room Temp.
<b>Zymo-Spin™ IIC-XL Columns</b>	10 columns	50 columns	200 columns	Room Temp.
<b>Collection Tubes</b>	20 tubes	100 tubes	400 tubes	Room Temp.
<b>Instruction Manual</b>	1	1	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 maximal performance and reliability.

\* The **Solid Tissue Buffer (Blue)** and **DNA Pre-Wash Buffer** may have formed a precipitate. If this is the case, incubate at 37°C to solubilize. DO NOT MICROWAVE.

## Specifications

- **Sample Sources** – See pages 2 and 3.
- **Workflow Overview** – Utilizes a Proteinase K Digestion and Zymo-Spin™ Technology for effective recovery of DNA. See page 5 for more information.
- **DNA Types** – The **Quick-DNA™ Miniprep Plus Kit** will isolate total DNA including genomic, mitochondrial, plasmid, viral, parasitic, etc. from biological fluids, cultured/monolayer cells, or solid tissues. Not recommended for small, cell-free DNA isolation from urine and serum/plasma (see specialized kits D3061 & D4076 respectively).
- **DNA Purity** - High quality DNA is ready for all sensitive downstream applications such as PCR, endonuclease digestion, Southern blotting, genotyping, Next-Generation Sequencing, bisulfite conversion, etc. ( $A_{260}/A_{230} \geq 2.0$ ).
- **DNA Size** - Capable of recovering genomic and mitochondrial DNA sized fragments > 50 kb. If present, parasitic, microbial, and viral DNA will also be recovered.
- **DNA Yield** - The DNA binding capacity of the column is 25 µg. Typically, mammalian tissues yield: 1-3 µg DNA per mg skeletal, heart, lung, and brain tissues and 3-5 µg DNA per mg liver and kidney. Human whole blood will yield 3-7 µg DNA per 100 µl blood sampled.
- **Elution Volume** - DNA can be eluted into as little as 35 µl **DNA Elution Buffer** or water.
- **Equipment** - Water bath or heat block (55°C), microcentrifuge, and vortex.
- **DNA Applications** – DNA isolated using the **Quick-DNA™ Miniprep Plus Kit** can be used for life-science research, genotyping, livestock breeding, veterinary research, and routine applied testing among a variety of other applications.

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.

## ZYMO RESEARCH CORP.

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## **Sample Sources**

**Biological Fluids:** For total DNA isolation from  $\leq 200 \mu\text{l}$  of whole blood, nucleated blood, buffy coat, saliva, sputum, semen, milk, etc.

### *Special Considerations*

- For biological fluids samples stored in DNA/RNA Shield™, see Appendix B (pg. 8).
- For nucleated blood samples, such as avian blood, see Appendix C (pg. 9).
- For blood, saliva, and cells collected onto Guthrie, FTA®, and other storage papers (cards), see Appendix F (pg. 12)
- For viral DNA isolation from serum/plasma samples, follow the Biological Fluids & Cells workflow. For small, cell-free DNA isolation from serum/plasma, use the Quick-cfDNA™ Serum & Plasma Kit (D4076).
- To isolate cellular and/or cell-free DNA from up to 40 ml of urine samples, see the Quick-DNA™ Urine Kit (D3061). For cellular DNA from urine, pellet at  $3,000 \times g$  for 15 minutes and remove supernatant before processing using the Biological Fluids & Cells workflow.

**Mammalian/Insect Cell Cultures:** For total DNA isolation from  $\leq 5 \times 10^6$  cells such as HeLa cells, HEK-293 cells, *Drosophila* cell lines, etc.

### *Special Considerations*

- Media should be removed before processing by pelleting cells (at approximately  $500 \times g$  for 2 minutes depending on volume and cell type) and removing the supernatant.
- For mammalian cell samples, it is possible to reduce Proteinase K digestion time to 5 minutes at  $55^\circ\text{C}$  (Step 2 on pg. 6).
- For cell monolayer and buccal cell preparation and collection, see Appendix A. (pg. 7).
- For samples stored in DNA/RNA Shield™, see Appendix B (pg. 8).

**Bacterial Cell Cultures:** For total DNA isolation (e.g. genomic, plasmid, etc.) from  $\leq 5 \times 10^6$  *E. coli* cells.

### *Special Considerations*

- Media should be removed before processing by pelleting cells (at approximately  $500 \times g$  for 2 minutes depending on volume and cell type) and removing the supernatant.
- For *E. coli* samples, follow the Biological Fluids & Cells workflow. All other bacterial samples may be resistant to chemical lysis and Proteinase K digestion and should be processed using the ZymoBIOMICS™ DNA Miniprep Kit (D4300).

**Solid Tissues:** For total DNA isolation from  $\leq 25$  mg tail snips, ear punches, organ biopsies (brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.).

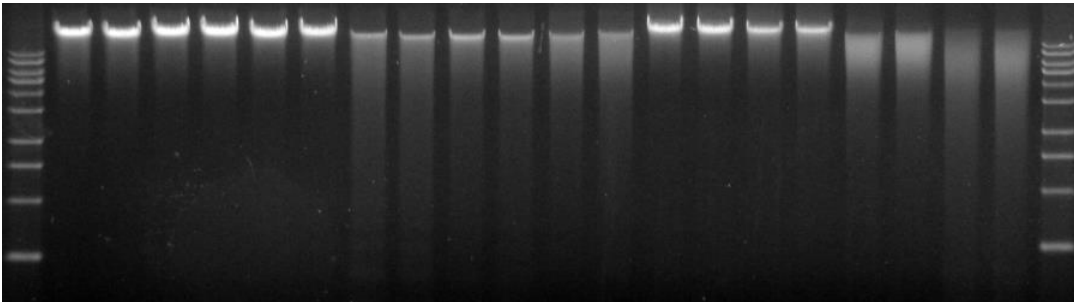
*Special Considerations*

- Overnight Proteinase K digestion at 55°C is possible (Step 2, **pg. 6**).
- For solid tissue samples stored in DNA/RNA Shield™, see Appendix B (**pg. 8**).
- For hair and feather samples, see Appendix D (**pg. 10**).
- For FFPE samples, see the *Quick-DNA*™ FFPE Kit (D3067) for specialized FFPE DNA purification. See Appendix E (**pg. 11**) for an adapted protocol using the *Quick-DNA*™ Miniprep Plus Kit.

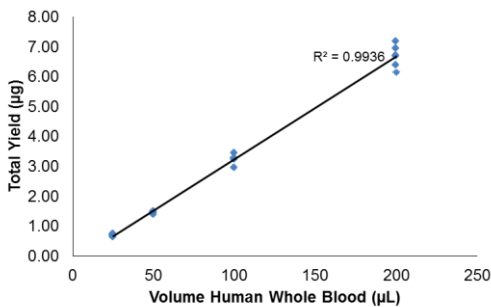
## Product Description

The **Quick-DNA™ Miniprep Plus Kit** is the easiest method for high yield total DNA extraction (e.g., genomic, plasmid, mitochondrial, viral) from any biological fluid, cell culture, or solid tissue sample. Innovative reagents and Zymo-Spin™ Technology allow for ultra-pure and concentrated genomic DNA > 50 kb to be eluted in as little as 35 µl. Zymo-Spin™ Columns ensure no buffer retention. Purified DNA is RNA-free, bypassing the need for RNase A treatment and ensuring accurate quantification for applications like library preparations. Isolated DNA is suitable for immediate use in sensitive downstream applications including qPCR, DNA-seq, arrays, and methylation analysis.

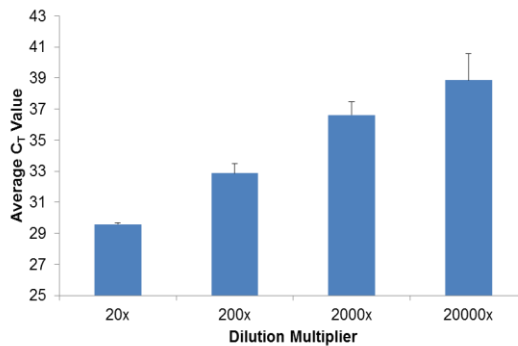
M Human Blood Porcine Blood HeLa Cells Buccal Swab Human Saliva Mouse Tail Mouse Kidney Mouse Brain Bovine Muscle Bovine Milk M



**High Quality DNA Obtained from a Wide Range of Biological Samples Using the Quick-DNA™ Miniprep Plus Kit.** DNA purified using the Quick-DNA™ Miniprep Plus Kit is ultrapure, highly concentrated, and ready for all downstream applications. Input DNA was standardized to 300 ng and analyzed in a 1% (w/v) TAE/agarose/EtBr gel (shown above). The size marker "M" is a 1 kb ladder (Zymo Research).



**DNA Yields Increase Linearly with Increasing Volumes of Human Whole Blood Using the Quick-DNA™ Miniprep Plus Kit.** Six replicates of 25, 50, 100, and 200 µl of human whole blood were processed.



**HSV-1 Viral DNA is Effectively Isolated from Plasma Using the Quick-DNA™ Miniprep Plus Kit.** A dilution series of HSV-1 spiked into porcine plasma and extracted using the Quick-DNA™ Miniprep Plus Kit shows effective purification and subsequent qPCR amplification, even at a 20,000:1 dilution. The no template controls did not amplify even after 50 cycles.

The **Quick-DNA™ 96 Plus Kit** (D4070, D4071) provides high-throughput (i.e., 96-well plate) processing of biological fluid, cell culture, and solid tissue samples.

For routine plasmid DNA purification from *E. coli*, Zymo Research offers the **Zyppy™ Plasmid Miniprep Kit** (D4036) and the **ZymoPURE™ Midi, Maxi, and Gigaprep Kits** (D4200, D4202, and D4204).

Zymo Research offers the **EZ DNA Methylation-Lightning™ Kit** (D5030, D5031) for rapid, precise DNA methylation detection and a comprehensive selection of other epigenetic tools.

Looking to isolate RNA? For RNA isolation from TRIzol®, the **Direct-zol™ RNA Miniprep Plus Kits** (R2070, R2072, R2071, R2073) offer total RNA purification without phase separation in only 7 minutes!

**Purification Guide**

The **Quick-DNA™ Miniprep Plus Kit** facilitates rapid and efficient purification of DNA from any biological fluids, cultured/monolayer cells, or solid tissues by combining enzymatic and chemical extraction regimens.

**Quick-DNA™ Miniprep Plus Kit Workflow**

**Biological Fluids & Cells**

**Biological Fluids: ≤ 200 µl**

Whole blood, nucleated blood, semen, buffy coat, saliva, body fluids, milk, etc.\*

Blood, saliva, and cells collected on storage paper/cards (Appendix F).

**Cultured Cells: ≤ 5 x 10<sup>6</sup>**

*E. coli*, insect, or mammalian cells (e.g. HeLa cells, buccal cells, HEK-293 cells, *Drosophila* cells, etc.).

**Solid Tissues**

**Solid Tissues: ≤ 25 mg**

Tail snips, ear punches, organ biopsies (Brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.).

FFPE samples (Appendix E).

Hair and feather samples (Appendix D).

BioFluid & Cell Buffer (Red)

Solid Tissue Buffer (Blue)

Proteinase K Digestion at 55°C

Genomic Binding Buffer

Spin Wash Elute } Zymo-Spin™ Technology

Ultra-Pure DNA

\* Viral DNA from serum or plasma samples can also be processed using this workflow. Not recommended for cell-free DNA isolation from urine, serum, or plasma samples.

For cell-free DNA isolation from up to 40 ml urine, see the **Quick-DNA™ Urine Kit** (D3061). For cell-free DNA isolation from up to 10 ml serum or plasma samples, see the **Quick-cfDNA™ Serum & Plasma Kit** (D4076).

## Reagent Preparation

- ✓ Add 1,060  $\mu$ l **Proteinase K Storage Buffer** to each **Proteinase K** (20 mg) tube prior to use. The final concentration of **Proteinase K** is  $\sim$ 20 mg/ml. Store at  $-20^{\circ}\text{C}$  after mixing.

## Protocol

Resuspend cultured cell or *E. coli* pellets using **DNA Elution Buffer** or an isotonic buffer (e.g. PBS):  
 $< 1 \times 10^6$  cells in 100  $\mu$ l  
 $1-5 \times 10^6$  cells in 200  $\mu$ l

Overnight Proteinase K digestions at  $55^{\circ}\text{C}$  are possible without affecting the integrity of the DNA.

### Biological Fluids & Cells

1. Add up to 200  $\mu$ l sample to a microcentrifuge tube and add:  
 200  $\mu$ l **BioFluid & Cell Buffer (Red)**  
 20  $\mu$ l **Proteinase K**
- Note:** For inputs  $< 200 \mu\text{l}^1$  biological fluid, proportionally decrease BioFluid & Cell Buffer (Red), Proteinase K, and Genomic Binding Buffer.
2. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at  $55^{\circ}\text{C}$  for 10 minutes.
  3. Add 1 volume **Genomic Binding Buffer** to the digested sample. Mix thoroughly or vortex 10-15 seconds.

**Example:** Add 420  $\mu$ l Genomic Binding Buffer to the 420  $\mu$ l digested sample.

### Solid Tissues

1. To a tissue sample ( $\leq 25$  mg) in a microcentrifuge tube, add a solution of:  
 95  $\mu$ l Water  
 95  $\mu$ l **Solid Tissue Buffer (Blue)**  
 10  $\mu$ l **Proteinase K**
  2. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at  $55^{\circ}\text{C}$  for 1-3 hours or until tissue solubilizes. Mix thoroughly before proceeding.
- Note:** To remove insoluble debris, centrifuge at  $\geq 12,000 \times g$  for 1 minute. Transfer aqueous supernatant<sup>2</sup> to a clean microcentrifuge tube.
3. Add 2 volumes **Genomic Binding Buffer** to the supernatant. Mix thoroughly or vortex 10-15 seconds.

**Example:** Add 400  $\mu$ l Genomic Binding Buffer to the 200  $\mu$ l supernatant.

4. Transfer the mixture to a **Zymo-Spin™ IIC-XL Column** in a **Collection Tube**. Centrifuge at  $\geq 12,000 \times g$  for 1 minute<sup>3</sup>. Discard the collection tube with the flow through.
5. Add 400  $\mu$ l **DNA Pre-Wash Buffer** to the spin column in a new **Collection Tube**. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
6. Add 700  $\mu$ l **g-DNA Wash Buffer** to the spin column. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
7. Add 200  $\mu$ l **g-DNA Wash Buffer** to the spin column. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Discard the collection tube with the flow through.
8. Transfer the spin column to a clean microcentrifuge tube. Add  $\geq 50 \mu\text{l}^4$  **DNA Elution Buffer** or water<sup>5</sup> directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at maximum speed for 1 minute to elute the DNA<sup>6</sup>. The eluted DNA can be used immediately for molecular based applications or stored  $\leq -20^{\circ}\text{C}$  for future use.

#### Notes:

<sup>1</sup> If using  $< 50 \mu\text{l}$  sample, increase the volume to 50  $\mu$ l using **DNA Elution Buffer** or an isotonic buffer (PBS) before continuing.

<sup>2</sup> Avoid transferring lipid layer and pelleted cellular debris.

<sup>3</sup> If the lysate is still visible on top of the matrix, centrifuge for another minute or until completely cleared.

<sup>4</sup> You can elute in as little as 35  $\mu$ l for highly concentrated DNA. See Figure on **pg. 16** for more information.

<sup>5</sup> **DNA Elution Buffer:** 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, ensure the pH is  $> 6.0$ .

<sup>6</sup> The total yield can be improved by eluting the DNA with  $60-70^{\circ}\text{C}$  **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

## **Appendix A**

### **Cell Monolayer Sample Preparation:**

*The following procedure is designed for up to  $5 \times 10^6$  monolayer cells. Although cell types and culture conditions may vary, the protocol will work with high-density growth cells (e.g., HeLa cells) as well as with low-density growth cells (e.g., neuronal cells).*

Trypsinize or scrape adherent cells from a culture flask or plate. Centrifuge the suspension at approximately 500 x g for 5 minutes. Remove the supernatant and resuspend the cell pellet in 1 ml PBS (Phosphate Buffered Saline) and then transfer suspension to a microcentrifuge tube. Centrifuge the suspension at approximately 500 x g for 5 minutes. Discard the supernatant and then follow the Biological Fluids & Cells workflow on Page 6.

### **Guidelines for Monolayer Cell DNA Isolation:**

Cell numbers (growth densities) can vary between different cell types. Table 1 (below) provides an approximation of the cell numbers that can be recovered from different culture containers for “high-density” growth cells like CV1 and HeLa cells.

**Table 1: Culture Plate/Flask Growth Area (cm<sup>2</sup>) and Cell Number**

<b>Culture Container</b>	<b>Well /Flask Surface Area</b>	<b>Cell Number</b>
96-well plate	0.32-0.6 cm <sup>2</sup>	4-5 x 10 <sup>4</sup>
24-well plate	2 cm <sup>2</sup>	1-3 x 10 <sup>5</sup>
12-well plate	4 cm <sup>2</sup>	4-5 x 10 <sup>5</sup>
6-well plate	9.5 cm <sup>2</sup>	0.5-1 x 10 <sup>6</sup>
T25 Culture Flask	25 cm <sup>2</sup>	2-3 x 10 <sup>6</sup>
T75 Culture Flask	75 cm <sup>2</sup>	0.6-1 x 10 <sup>7</sup>
T175 Culture Flask	175 cm <sup>2</sup>	2-3 x 10 <sup>7</sup>

### **Buccal Cells and Swabs:**

*Buccal cells can be isolated using a rinse- or swab-based isolation method.*

**A. Rinse Method:** Vigorously rinse mouth with 10-20 ml of saline solution or mouthwash orally for 30 seconds. The more vigorous the rinsing action, the more cells that will be recovered. Spit the saline into a 50 ml tube and pellet the cells at 1,500 rpm for 5 minutes. Discard the supernatant without disturbing the cell pellet. Continue from Step 1 of the Biological Fluids & Cells workflow on Page 6.

**B. Swab Isolation Method:** Thoroughly rinse mouth with water before isolating cells. Brush the inside of the cheek with a buccal swab for 15 seconds (approximately 20 brushes), making sure to cover the entire area of the inner cheek. Rinse the brush into a microcentrifuge tube using a mixture of 200 µl of **BioFluid & Cell Buffer (Red)** and 200 µl **DNA Elution Buffer** or another isotonic solution. Add 20 µl of **Proteinase K**, mix thoroughly, and incubate at 55°C for 10 minutes. Continue from Step 3 of the Biological Fluids & Cells workflow on Page 6.



## **Appendix B**

### **Samples in DNA/RNA Shield™:**

*DNA/RNA Shield™ ensures nucleic acid stability during sample storage/transport at ambient temperatures. There is no need for refrigeration or specialized equipment. DNA/RNA Shield™ effectively lyses cells and inactivates nucleases and infectious agents (virus), and it is compatible with various collection and storage devices (vacutainers, swabs, nasal, buccal, fecal etc.).*

*DNA/RNA Shield™ purchased separately (R1100 or R1200).*

### **Biological Fluids and Cell Cultures**

1. Add 20 µl of Proteinase K to 400 µl of the sample/shield mixture prepared according to the DNA/RNA Shield™ specifications.
2. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at room temperature for 20 minutes.
3. Continue from Step 3 of the Biological Fluids & Cells Workflow (**pg. 6**).

### **Solid Tissues**

1. To each 300 µl sample prepared according the DNA/RNA Shield™ specifications, add 150 µl **Solid Tissue Buffer (Blue)** and 10 µl Proteinase K.
2. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at 55°C 1 – 3 hours.

**Note:** Overnight digestion at 55°C is possible and will increase the effectiveness of digestion and DNA recoveries.

3. To remove insoluble debris, centrifuge at  $\geq 12,000 \times g$  for 1 minute. Transfer aqueous supernatant to a clean microcentrifuge tube.
4. Add 1 volume **Genomic Binding Buffer** to the digested sample. Mix thoroughly or vortex 10-15 seconds.
5. Continue from Step 4 of the main protocol (**pg. 6**).

**Notes:**

<sup>1</sup> If the lysate is still visible on top of the matrix, centrifuge for another minute or until completely cleared.

<sup>2</sup> You can elute in as little as 35  $\mu$ l for highly concentrated DNA. See Figure on pg. 16 for more information.

<sup>3</sup> **DNA Elution Buffer:**  
10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

<sup>4</sup> The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

**Appendix C****Nucleated Blood Samples**

1. Add up to 10  $\mu$ l of nucleated blood to the following:

<b>BioFluid &amp; Cell Buffer (Red)</b>	200 $\mu$ l
<b>Proteinase K</b>	20 $\mu$ l
<b>DNA Elution Buffer (or TE Solution)</b>	200 $\mu$ l

2. Mix thoroughly by pipetting up and down. Then incubate the tube at 55°C for 20 minutes.

**Note:** The sample may not be completely homogenous before digesting.

3. Add 1 volume of **Genomic Binding Buffer** to the tube and mix thoroughly by pipetting up and down and by vortexing. Ensure the sample is homogenous before continuing.

**Note:** It may be necessary to pipette up and down many times to ensure the sample is homogenous. Vortexing will also help ensure the mixture is homogenous.

4. Transfer the mixture to a **Zymo-Spin™ IIC-XL Column** in a **Collection Tube**. Centrifuge at  $\geq 12,000 \times g$  for 1 minute<sup>1</sup>. Discard the collection tube with the flow through.
5. Add 400  $\mu$ l **DNA Pre-Wash** to the spin column in a **new Collection Tube**. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
6. Add 700  $\mu$ l **g-DNA Wash Buffer** to the spin column. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
7. Add 200  $\mu$ l **g-DNA Wash Buffer** to the spin column directly on the matrix. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Discard the collection tube with the flow through.
8. Transfer the spin column to a clean microcentrifuge tube. Add  $\geq 50 \mu$ l<sup>2</sup> **DNA Elution Buffer** or water<sup>3</sup> directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at top speed for 1 minute to elute the DNA<sup>4</sup>. The eluted DNA can be used immediately for molecular based applications or stored  $\leq -20^\circ\text{C}$  for future use.

## **Appendix D**

### **Hair and Feather Samples:**

1. Freshly prepared DTT (dithiothreitol) (not provided) needs to be added to the Proteinase K Digestion and sample ( $\leq 25$  mg) as follows:

Water	90 $\mu$ l
<b>Solid Tissue Buffer (Blue)</b>	90 $\mu$ l
DTT (1 M)	10 $\mu$ l
Proteinase K	10 $\mu$ l

2. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at 55°C for 1-3 hours.

**Note:** Overnight digestions are possible without affecting the integrity of the DNA.

3. Add 400  $\mu$ l **Genomic Binding Buffer** to the tube and mix thoroughly by vortexing for 15 seconds. Centrifuge at  $\geq 12,000 \times g$  for 1 minute to pellet insoluble debris.
4. Transfer the mixture (supernatant) to a **Zymo-Spin™ IIC-XL Column** in a **Collection Tube**. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Discard the collection tube with the flow through.
5. Add 400  $\mu$ l **DNA Pre-Wash** to the spin column in a new **Collection Tube**. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
6. Add 700  $\mu$ l **g-DNA Wash Buffer** to the spin column. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
7. Add 200  $\mu$ l **g-DNA Wash Buffer** to the spin column directly on the matrix. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Discard the collection tube with the flow through.
8. Transfer the spin column to a clean microcentrifuge tube. Add  $\geq 50 \mu$ l<sup>1</sup> **DNA Elution Buffer** or water<sup>2</sup> directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at top speed for 1 minute to elute the DNA<sup>3</sup>. The eluted DNA can be used immediately for molecular based applications or stored  $\leq -20^\circ\text{C}$  for future use.

#### **Notes:**

<sup>1</sup> You can elute in as little as 35  $\mu$ l for highly concentrated DNA. See Figure on **pg. 16** for more information.

<sup>2</sup> **DNA Elution Buffer:**  
10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is  $> 6.0$ .

<sup>3</sup> The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Alternatively, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

**Notes:**

The **Quick-DNA™ FFPE Kit** (D3067) is specialized for DNA purification from FFPE samples.

<sup>1</sup> It is possible to store samples at -80°C at this point for later use.

<sup>2</sup> If a ≤ 25 mg tissue sample is not fully submerged in the digestion volume, scale up the digestion to 200 µl while keeping the amount of **Proteinase K** the same.

<sup>3</sup> You can elute in as little as 35 µl for highly concentrated DNA. See Figure on **pg. 16** for more information.

<sup>4</sup> **DNA Elution Buffer:**  
10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

<sup>5</sup> The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

**Appendix E****FFPE Samples:****Deparaffinize FFPE Samples:**

1. Remove or trim as much paraffin from the sample(s) as possible (≤ 25 mg).
2. Transfer samples to 1.5 ml microcentrifuge tubes. Add 750 µl xylene (not provided) to the samples.
3. Vortex and incubate samples at room temperature for 1 hour with gentle rocking.
4. Centrifuge at 12,000 x g for 1 minute and remove the xylene from the sample. Repeat steps 2-4.
5. Wash with 1 ml ethanol (100%), vortex briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 12,000 x g for 1 minute, discard the supernatant, and repeat.
6. Wash with 1 ml ethanol (95%), vortex briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 12,000 x g for 1 minute, discard the supernatant, and repeat.
7. Wash with 1 ml ethanol (75%), vortex briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 12,000 x g for 1 minute, discard the supernatant, and repeat.
8. Wash with 1 ml ddiH<sub>2</sub>O, vortex briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 12,000 x g for 1 minute and remove the water from the sample<sup>1</sup>.

**DNA Extraction:**

1. Prepare the Proteinase K Digestion to the deparaffinized samples as follows<sup>2</sup>:
 

Water	45 µl
<b>Solid Tissue Buffer (Blue)</b>	45 µl
Proteinase K	10 µl
2. Mix thoroughly or vortex 10-15 seconds and incubate the tube at 55°C for 12-16 hours. Then incubate the tube at 94°C for 20 minutes.
3. Add 6 volumes **Genomic Binding Buffer** to the tube and mix thoroughly by vortexing for 15 seconds. Centrifuge at ≥ 12,000 x g for 1 minute to pellet insoluble debris.
4. Transfer the mixture (supernatant) to a **Zymo-Spin™ IIC-XL Column** in a **Collection Tube**. Centrifuge at ≥ 12,000 x g for 1 minute. Discard the collection tube with the flow through.
5. Add 400 µl **DNA Pre-Wash Buffer** to the spin column in a new **Collection Tube**. Centrifuge at ≥ 12,000 x g for 1 minute. Empty the collection tube.
6. Add 700 µl **g-DNA Wash Buffer** to the spin column. Centrifuge at ≥ 12,000 x g for 1 minute. Empty the collection tube.
7. Add 200 µl **g-DNA Wash Buffer** to the spin column directly on the matrix. Centrifuge at ≥ 12,000 x g for 1 minute. Discard the collection tube with the flow through.
8. Transfer the spin column to a clean microcentrifuge tube. Add ≥ 50 µl<sup>3</sup> **DNA Elution Buffer** or water<sup>4</sup> directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at top speed for 1 minute to elute the DNA<sup>5</sup>. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20°C for future use.

## Appendix F

### Samples Collected onto Storage Papers/Cards:

Rapid purification of inhibitor-free, PCR-quality DNA from blood, saliva, and cells collected onto Guthrie, FTA®, and other storage papers (cards). The procedure is easy; card punches are added directly to a ZR BashingBead™ Lysis Tube (2.0 mm) and thoroughly homogenized by bead beating (e.g. FastPrep®-24, or similar). Following Proteinase K digestion, the DNA is purified using innovative Zymo-Spin™ Technology. Eluted DNA is ideal for PCR, genotyping, etc.

ZR BashingBead Lysis Tubes (2.0 mm) (S6003-50) and Lysis Solution (D6001-3) purchased separately.

1. Add card samples (punches) to a **ZR BashingBead™ Lysis Tube (2.0 mm)**<sup>1</sup>. Add 400 µl **Lysis Solution**<sup>2</sup> to the tube.
2. Secure lysis tube in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed.

**Note:** Processing times may be as little as 40 seconds when using high-speed disrupters (e.g., FastPrep®-24, or similar). See manufacturer's literature for operating instructions.

3. Centrifuge the **ZR BashingBead™ Lysis Tube (2.0 mm)** at  $\geq 10,000 \times g$  for 1 minute.
4. To the lysate in the **ZR BashingBead™ Lysis Tube (2.0 mm)**, add:

<b>Proteinase K</b>	40 µl
<b>Solid Tissue Buffer (Blue)</b>	360 µl

5. Mix and then incubate the tube at 55°C for 10-15 minutes.
6. Centrifuge the **ZR BashingBead™ Lysis Tube (2.0 mm)** at  $\geq 10,000 \times g$  for 1 minute. Transfer 400 µl supernatant to a microcentrifuge tube.
7. Add 800 µl **Genomic Binding Buffer** to the tube and mix thoroughly.
8. Transfer 600 µl of the mixture to a **Zymo-Spin™ IIC-XL Column** in a **Collection Tube**. Centrifuge at  $\geq 12,000 \times g$  for 1 minute.
9. Discard the flow through from the Collection Tube and repeat Step 8.
10. Add 400 µl **DNA Pre-Wash Buffer** to the spin column in a new Collection Tube. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
11. Add 700 µl **g-DNA Wash Buffer** to the spin column. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Empty the collection tube.
12. Add 200 µl **g-DNA Wash Buffer** to the spin column. Centrifuge at  $\geq 12,000 \times g$  for 1 minute. Discard the collection tube with the flow through.
13. Transfer the spin column to a clean microcentrifuge tube. Add  $\geq 50 \mu\text{l}$ <sup>3</sup> **DNA Elution Buffer** or water directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at maximum speed for 1 minute to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored  $\leq -20^\circ\text{C}$  for future use.

<sup>1</sup>**ZR BashingBead Lysis Tubes** (2.0 mm) (Catalog No. S6003-50)

<sup>2</sup>**Lysis Solution** (Catalog No. D6001-3-40 & D6001-3-150)

<sup>3</sup> **DNA Elution Buffer:**  
10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is  $> 6.0$ .

<sup>4</sup>The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

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# Quick-DNA™ Miniprep Plus Kit

## Quick Protocol

Catalog Nos. D4068 & D4069



### Biological Fluids & Cells Protocol

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#### Biological Fluids: ≤ 200 µl

Total DNA from whole blood, buffy coat, saliva, sputum, semen, etc. See the Instruction Manual page 2 for special considerations.

#### Cultured Cells: ≤ 5x10<sup>6</sup>

Total DNA from *E. coli*, insect, or mammalian cells (e.g. HeLa cells, buccal cells, HEK-293 cells, etc.). See the Instruction Manual page 2 for special considerations.

**Note:** Pellet cells and discard supernatant. Resuspend cell pellets using **DNA Elution Buffer** or an isotonic buffer (e.g. PBS):  
 < 1 x 10<sup>6</sup> cells in 100 µl  
 1-5 x 10<sup>6</sup> cells in 200 µl

\*Add 1,060 µl of Storage Buffer to each 20 mg tube of Proteinase K. Store at -20°C.

1. Add up to 200 µl sample to a microcentrifuge tube and add:  
 200 µl **BioFluid & Cell Buffer (Red)**  
 20 µl **Proteinase K**

**Note:** For an input of less than 200 µl Biological Fluid, proportionally decrease BioFluid & Cell Buffer (Red), Proteinase K, and Genomic Binding Buffer.

2. Mix thoroughly and then incubate the tube at 55°C for 10 minutes.
3. Add 1 volume **Genomic Binding Buffer** to the digested sample. Mix thoroughly.  
**Example:** Add 420 µl Genomic Binding Buffer to the 420 µl digested sample.
4. Transfer the mixture to a **Zymo-Spin™ IIC-XL Column** in a **Collection Tube**. Centrifuge (≥ 12,000 x g) for 1 minute. Discard the collection tube with the flow through.
5. Add 400 µl **DNA Pre-Wash Buffer** to the column in a new Collection Tube and centrifuge for 1 minute. Empty the Collection Tube.
6. Add 700 µl **g-DNA Wash Buffer** and centrifuge for 1 minute. Empty the Collection Tube.
7. Add 200 µl **g-DNA Wash Buffer** and centrifuge for 1 minute. Discard the collection tube with the flow through.
8. To elute the DNA, transfer to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer** (minimum 35 µl), incubate for 5 minutes, and then centrifuge for 1 minute.

DNA  
Purification  
MADE SIMPLE  
Made Simple™

For the full Instruction Manual, visit  
<http://www.zymoresearch.com/m/D4068>

Ver. 1.2.0

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# Quick-DNA™ Miniprep Plus Kit

## Quick Protocol

Catalog Nos. D4068 & D4069



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*The Beauty of Science is to Make Things Simple*

## Solid Tissues Protocol

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### Solid Tissues: ≤ 25 mg

Total DNA from tail snips, ear punches, organ biopsies (brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.).

For special sample types including FFPE, hair and feather, see the Instruction Manual page 3.

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*\*Add 1,060 µl of Storage Buffer to each 20 mg tube of Proteinase K. Store at -20°C.*

1. To a tissue sample (≤ 25 mg) in a microcentrifuge tube, add a solution of:
  - 95 µl Water
  - 95 µl **Solid Tissue Buffer (Blue)**
  - 10 µl **Proteinase K**
2. Mix thoroughly and then incubate the tube at 55°C for 1-3 hours or until tissue solubilizes. Mix thoroughly.  
*Note: To remove insoluble debris, pellet by centrifugation at ≥ 12,000 x g for 1 minute. Transfer aqueous supernatant to a clean tube.*
3. Add 2 volumes **Genomic Binding Buffer** to the supernatant. Mix thoroughly.  
*Example: Add 400 µl Genomic Binding Buffer to the 200 µl supernatant.*
4. Transfer the mixture to a **Zymo-Spin™ IIC-XL Column** in a **Collection Tube**. Centrifuge (≥ 12,000 x g) for 1 minute. Discard the collection tube with the flow through.
5. Add 400 µl **DNA Pre-Wash Buffer** to the column in a new Collection Tube and centrifuge for 1 minute. Empty the Collection Tube.
6. Add 700 µl **g-DNA Wash Buffer** and centrifuge for 1 minute. Empty the Collection Tube.
7. Add 200 µl **g-DNA Wash Buffer** and centrifuge for 1 minute. Discard the collection tube with the flow through.
8. To elute the DNA, transfer to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer** (minimum 35 µl), incubate for 5 minutes, and then centrifuge for 1 minute.

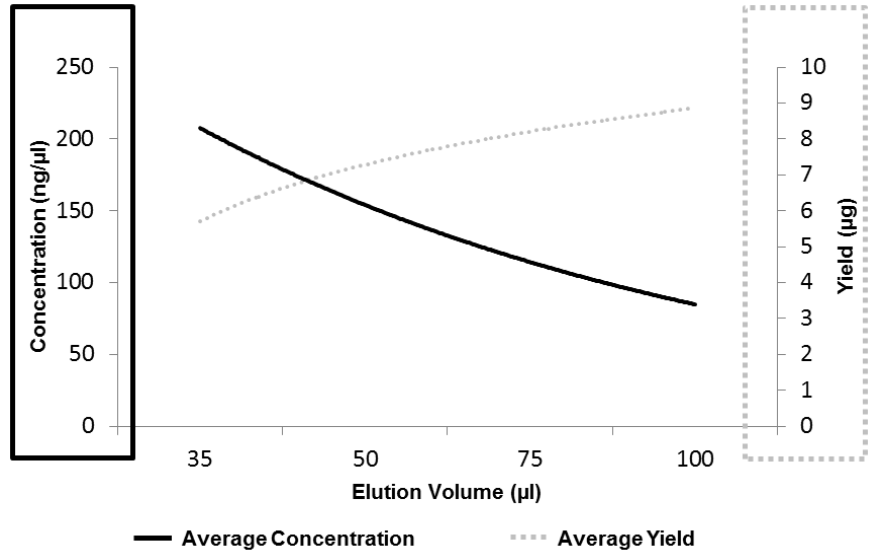
**Troubleshooting:**

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

Problem	Possible Causes and Suggested Solutions
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**DNA Elution Guide**

Identifying Desired Elution Volume



**The Relationship Between Elution Volume, DNA Yield, and DNA Concentration Using Porcine Whole Blood.** Using a smaller elution volume results in higher concentrations of DNA samples, but with reduced yields. Using a larger elution volume results in higher DNA yields, but at a reduced concentration. Choose an elution volume that best fits your individual application.

Increasing DNA Yields

- The total yield may be improved by eluting the DNA with DNA Elution Buffer pre-heated to 60-70°C.
- Loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

**DNA Degradation**

DNase Contamination

- Check pipettes, pipette tips, microcentrifuge tubes, etc. for DNase contamination and exercise the appropriate precautions during the DNA purification procedure. All reagents and components supplied with the **Quick-DNA™ Miniprep Plus Kit** are DNase-free.
- If water is used to elute the DNA, ensure that DNase-free water is used.
- Certain samples are more prone to degradation as a result of the conditions used for storage and transport (e.g. FFPE Tissue).

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Incomplete Debris Removal

- For solid tissue samples, ensure lysate is centrifuged after digestion to pellet insoluble debris. Ensure pellet is not transferred to the column.

Incomplete Lysis/Digestion

- Ensure Proteinase K digestions are performed at 55°C as indicated. Extend digestion time to 20 minutes if samples are high in protein.
- Vortex samples longer after the addition of Genomic Binding Buffer to ensure that the lysate is homogenous.

Tissue Input

- For low DNA-containing tissues (e.g. muscle, etc.) using larger inputs will increase yields ( $\leq 25$  mg).
- If the lysate does not pass through the column or is extremely viscous, use less input material. Too much tissue can cause cellular debris to overload the column and result in recovery of dirty DNA.

**Low DNA Yield**

Elution Procedures

- Ensure the DNA Elution Buffer hydrates the matrix for 5 minutes at room temperature before centrifugation.
- To increase yields, heat the DNA Elution Buffer to 60-70°C before use. You can also load the eluate a second time, incubate at room temperature for 3 minutes, and centrifuge again.

Procedural Errors

- Ensure the proper digestion buffer is used. See the Purification Guide on page 5.
- Ensure the correct volume of Genomic Binding Buffer is used. For plasma and serum samples, use 3 volumes of Genomic Binding Buffer. See the Purification Guide on page 5 and the Protocol on page 6.

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Procedural Errors

- The column tip is contaminated with wash buffer flow through. Ensure the tip does not touch the flow through. Empty the collection tube or use a new collection tube when instructed.
- Insufficient centrifugation: Ensure the indicated centrifugation times and speeds are used. Increase the centrifugation time of the final wash step by one minute to ensure complete wash buffer removal.

**Low DNA Performance**

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**Low DNA Performance**  
*Continued*

Tissue Input

- Make sure the lysate has passed completely through the matrix before proceeding to the wash steps.
- If the lysate does not pass through the column or is extremely viscous, use less input material. Too much tissue can cause cellular debris to overload the column and leech salts into the DNA eluate.

RNA in Eluate

- All reagents and components supplied with the **Quick-DNA™ Miniprep Plus Kit** are designed for RNA removal. Typically if RNA is in the eluate, too much tissue/sample was used.
  - Ensure the proper amount of Genomic Binding Buffer and corresponding digestion buffer is used. See the Purification Guide on page 5.
  - Ensure Proteinase K digestions are performed at 55°C as indicated.
  - For applications sensitive to trace amounts of RNA, additional RNA removal may be necessary using an RNase A treatment.
-

### Ordering Information

Product Description	Catalog No.	Kit Size
<b>Quick-DNA™ Miniprep Plus Kit</b>	D4068T	10 preps.
	D4068	50 preps.
	D4069	200 preps.
<b>Quick-DNA™ Microprep Plus Kit</b>	D4074	50 preps.
<b>Quick-DNA™ 96 Plus Kit</b>	D4070	2 x 96 preps.
	D4071	4 x 96 preps.
For Individual Sale	Catalog No.	Amount
<b>Proteinase K &amp; Storage Buffer</b>	D3001-2-5	5 mg set
	D3001-2-20	20 mg set
<b>BioFluid &amp; Cell Buffer (Red)</b>	D4068-1-2.5	2.5 ml
	D4068-1-12	12 ml
	D4068-1-45	45 ml
<b>Solid Tissue Buffer (Blue)</b>	D4068-2-2.5	2.5 ml
	D4068-2-6	6 ml
	D4068-2-22	22 ml
<b>Genomic Binding Buffer</b>	D4068-3-5-S	5 ml
	D4068-3-25	25 ml
	D4068-3-85	85 ml
<b>DNA Pre-Wash Buffer</b>	D3004-5-15	15 ml
	D3004-5-30	30 ml
	D3004-5-50	50 ml
<b>g-DNA Wash Buffer</b>	D3004-2-50	50 ml
	D3004-2-100	100 ml
	D3004-2-200	200 ml
<b>DNA Elution Buffer</b>	D3004-4-4	4 ml
	D3004-4-10	10 ml
	D3004-4-50	50 ml
<b>Zymo-Spin™ IIC-XL Columns</b>	C1102-25	25 columns
	C1102-50	50 columns
<b>Collection Tubes</b>	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1,000 tubes

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