ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

DNA Purification *Made Simple*™

INSTRUCTION MANUAL

Quick-DNA™ Midiprep Plus Kit

Catalog No. D4075

Highlights

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

Contents

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

Product Contents

***Quick*-DNA™ Midiprep Plus Kit** (Kit Size)	**D4075** (25 Preps)	**Storage Temperature**
Proteinase K & Storage Buffer	3 x 20 mg	−20 °C (after mixing)
BioFluid & Cell Buffer (Red)	2 x 45 ml	Room Temp.
Solid Tissue Buffer (Blue)*	1 x 22 ml	Room Temp.
Genomic Lysis Buffer	1 x 150 ml	Room Temp.
DNA Pre-Wash Buffer *	1 x 250 ml	Room Temp.
g-DNA Wash Buffer	1 x 200 ml	Room Temp.
DNA Elution Buffer	1 x 10 ml	Room Temp.
Zymo-Spin™ V-E Columns with Reservoir	25	Room Temp.
Collection Tubes	50	Room Temp.
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 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** – Up to 3 ml of biological fluids, 3 x 10^7 mammalian/cell cultures, or 125 mg of solid tissue. See pages 2 and 3 for more information.
- **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™ Midiprep Plus Kit** will isolate total DNA including genomic, mitochondrial, plasmid, viral, parasitic, etc. 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 125 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 200 µl **DNA Elution Buffer** or water**.**
- **Equipment** - Water bath or heat block (55 °C), centrifuge or vacuum source and manifold, microcentrifuge, vortex, conical tubes (15 – 50 ml), and microcentrifuge tubes.
- **DNA Applications** – DNA isolated using the ***Quick*-DNA™ Midiprep Plus Kit** can be used for life-science research (*e.g.* Next-Gen Seq.), genotyping, livestock breeding, veterinary research, and routine applied testing among a variety of other applications.

Satisfaction of all Zymo Research products is guaranteed. If you are not satisfied with this product please call 1-888-882-9682.

All components are available separately, including: all reagents, plastics, and **Zymo-Spin™ V-E Columns with Reservoirs**.

Sample Sources

Biological Fluids: For total DNA isolation from ≤ 3 ml of human whole blood, highly nucleated blood, buffy coat, saliva, sputum, semen, milk, etc.

Special Considerations

- For biological fluids samples stored in DNA/RNA Shield™, see Appendix B.
- For highly nucleated blood samples, such as avian blood, see Appendix C.
- For viral DNA isolation from serum/plasma samples, follow the Biological Fluids & Cells workflow.
- For small cell-free DNA isolation from serum/plasma samples, use the *Quick*-cfDNA™ Serum & Plasma Kit (D4076).
- For cellular DNA from urine, pellet at 3,000 x *g* for 15 minutes and remove supernatant before processing with the Biological Fluids & Cells workflow. To isolate cellular and/or cell-free DNA from up to 40 ml of urine samples, see the *Quick*-DNA™ Urine Kit (D3061).

Mammalian/Insect Cell Cultures: For total DNA isolation from ≤ 3 x 10^7 cells such as HeLa cells, HEK-293 cells, *Drosophila* cell lines, etc.

Special Considerations

- Media should be removed before processing by pelleting cells (pellet cells at approximately 500 x *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 30 minutes at 55 °C (or until complete dissolution) (Step 2 on **pg. 6**).
- For cell monolayer and buccal cell preparation and collection, see Appendix A.
- For samples stored in DNA/RNA Shield™, see Appendix B.

Bacterial Cell Cultures: For total DNA isolation (e.g. genomic, plasmid, etc.) from ≤ 3 x 10^7 *E. coli* cells.

Special Considerations

- Media should be removed before processing by pelleting cells (pellet cells at approximately 500 x *g* for 2 minutes depending on volume and cell type) and removing the supernatant.
- For *E. coli* samples and other easy to lyse microbes*, follow the Biological Fluids & Cells workflow. All other bacterial samples may be resistant to chemical lysis and Proteinase K digestion and should be used with the *Quick*-DNA™ Fungal/Bacterial DNA Midiprep Kit (D6105).
- Microbes previously lysed with enzymes (e.g. Lysozyme) or other mechanical methods (e.g. bead beating or liquid nitrogen) may be processed by using the Biological Fluids & Cells workflow.

*Resistance to lysis to be determined by the researcher as *E. coli* is only a representative example.

Solid Tissues: For total DNA isolation from ≤ 125 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.
- For hair and feather samples, see Appendix D.
- For FFPE samples, see *Quick*-DNA™ FFPE Kit (D3067) for specialized FFPE DNA purification.

Environmental Sample Recommendations

- **Microbiomics and Metagenomics:** Use the ZymoBIOMICS® DNA Miniprep Kit (D4300) for accurate community profiling. The ZymoBIOMICS® DNA Miniprep Kit also includes innovative inhibitor removal technology enabling purification of inhibitor free DNA from nearly any sample type (feces, soil, water, biofilms etc.).
- **Microbial Isolation from Environmental Samples:** For samples not intended for community profiling, use the *Quick*-DNA™ Fecal/Soil Microbe Midiprep Kit (D6110).
- **Plants and Seeds:** Use the *Quick*-DNA™ Plant/Seed Miniprep Kit (D6020).

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

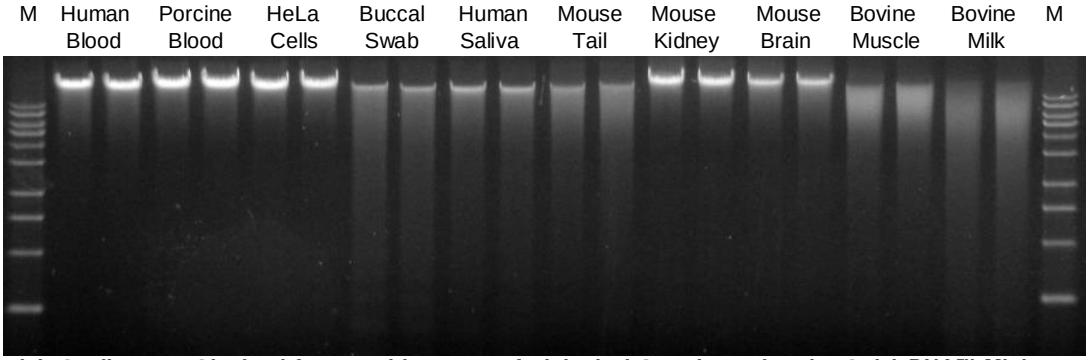
For routine plasmid DNA purification from *E. coli*, Zymo Research offers the **Zyppy™ Plasmid Miniprep Kit** (D4037) and the **ZymoPURE™ Plasmid Mini, Midi, Maxi,** and **Gigaprep Kits** (D4209, D4200, D4202, and D4204).

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 Plus Kits** (R2070, R2071, R2072, R2073) offer total RNA purification without phase separation in only 7 minutes!

Product Description

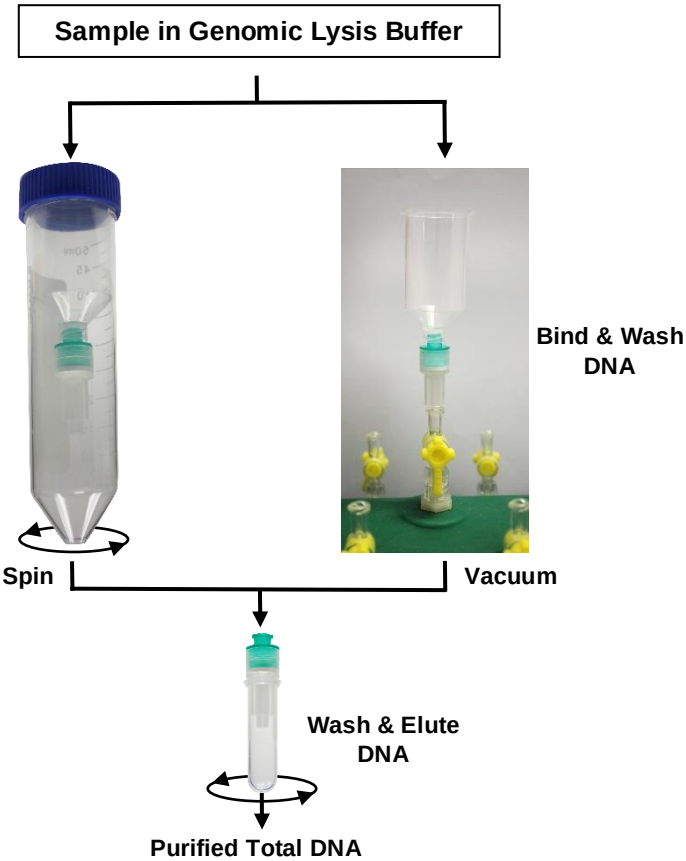
The ***Quick*-DNA™ Midiprep Plus Kit** is the easiest method for high yield total DNA extraction (e.g., genomic, plasmid, mitochondrial, viral) from any biological fluid, cell culture, or solid tissue sample. Innovative reagents and Zymo-Spin™ Technology allow for ultra-pure and concentrated genomic DNA > 50 kb to be eluted in as little as 200 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.



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).

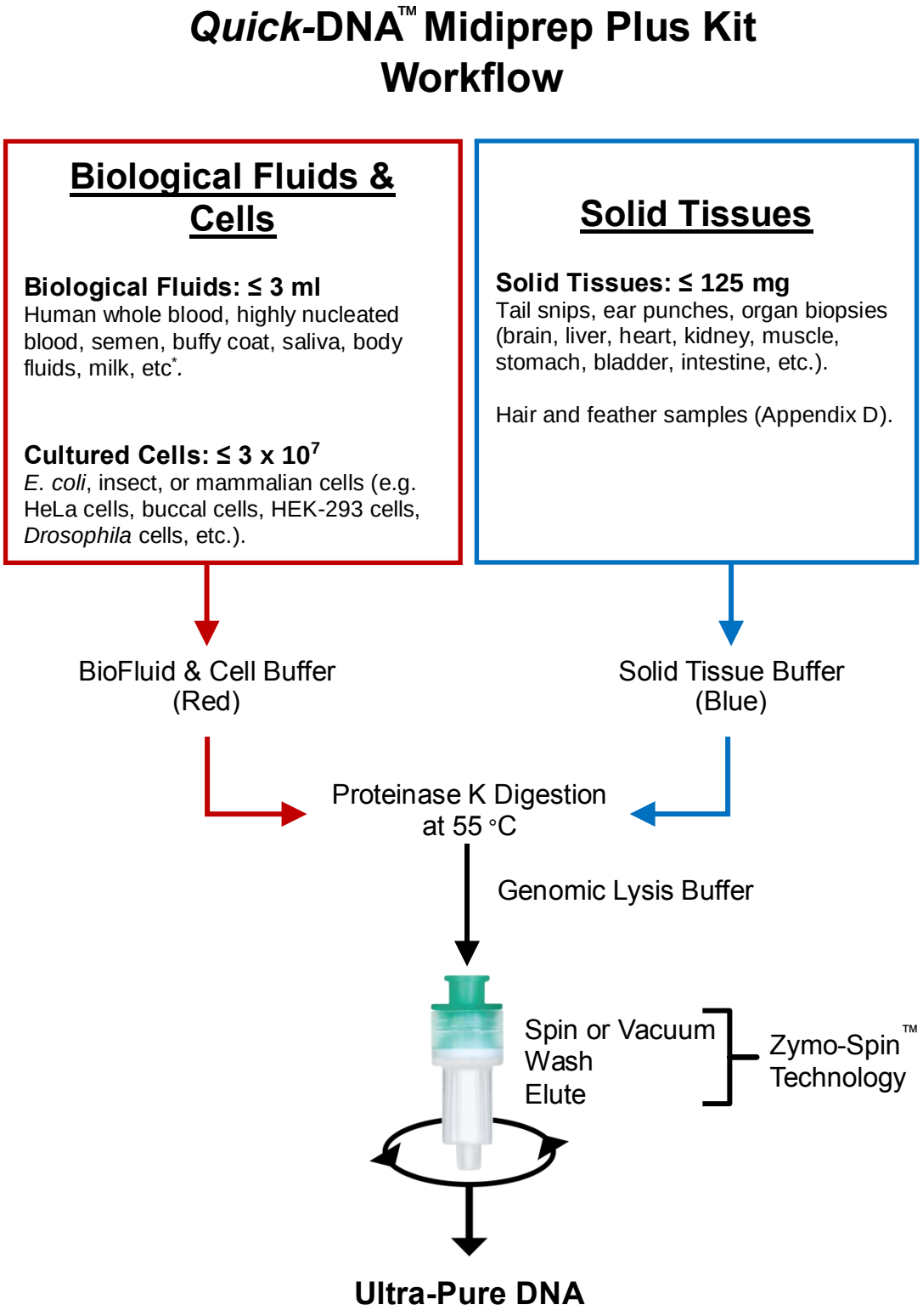
Zymo-Spin™ V-E Column with Reservoir

Zymo-Spin™ V-E Column



Purification Guide

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



Notes:

* 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).

Notes:

[1] All components are available separately, including: all reagents, plastics, and **Zymo-Spin™ V-E Columns with Reservoirs**.

Reagent Preparation

✓ Add 1,060 µl **Proteinase K Storage Buffer** to each **Proteinase K** (20 mg) tube prior to use[1]. The final concentration of **Proteinase K** is ~20mg/ml, after resuspension. Store at -20 °C after mixing.

Sample Preparation Protocol

*Resuspend cultured cells or E. coli pellets using **DNA Elution Buffer** or an isotonic buffer (e.g. PBS):*
< 1 x 10^6 cells in 500 µl
1-3 x 10^7 cells in 1,000 µl
Overnight Proteinase K digestions at 55 °C are possible without affecting the integrity of the DNA.

Biological Fluids & Cells

1. Add up to 3 ml sample to a 50 ml conical tube (not provided) and add:
   - 3 ml **BioFluid & Cell Buffer (Red)**
   - 100 µl **Proteinase K**

   ***Note:** For inputs < 3 ml biological fluid, proportionally decrease BioFluid & Cell Buffer (Red) and Proteinase K (See Table 1: Quick Setup Guide).*
2. Vortex for 15 seconds and then incubate the tube at 55 °C for:
   - 40 minutes if sample ≤ 1 ml
   - 2 hours if sample ≤ 3 ml
3. Add 1 volume **Genomic Lysis Buffer** to the digested sample, ignoring volume contribution from the Proteinase K.
   Vortex for 15 seconds (See Table 1: Quick Setup Guide).

   ***Example:** Add 6.0 ml Genomic Lysis Buffer to the 6.0 ml total volume digested sample.*

Table 1: Quick Setup Guide

Sample Volume	500 µl	1 ml	2 ml	3 ml
BioFluid & Cell Buffer (Red)	500 µl	1 ml	2 ml	3 ml
Proteinase K	20 µl	30 µl	70 µl	100 µl
Mix thoroughly and incubate at 55 °C for: 40 min if sample ≤ 1 ml or 2 hrs if sample ≤ 3 ml				
Add Genomic Lysis Buffer	1.0 ml	2.0 ml	4.0 ml	6.0 ml

Solid Tissues

1. To a tissue sample (≤ 125 mg) in a microcentrifuge tube (not provided), add a solution of:
   - 480 µl Water
   - 480 µl **Solid Tissue Buffer (Blue)**
   - 40 µl **Proteinase K**
2. Vortex for 15 seconds and then incubate the tube at 55 °C for 1-3 hours or until tissue solubilizes. Mix thoroughly before proceeding.

   ***Note:** To remove insoluble debris, centrifuge at 12,000 x g for 1 minute. Transfer aqueous supernatant to a 15 ml conical tube (not provided).*
3. Add 4 volumes **Genomic Lysis Buffer** to the supernatant. Vortex for 15 seconds.

   ***Example:** Add 4 ml Genomic Lysis Buffer to the 1 ml supernatant.*

DNA Purification Protocol

Centrifugation Protocol	*Vacuum Protocol*
1. Transfer the lysate to the **Zymo-Spin™ V-E Column/Reservoir** inserted in a 50 ml conical tube[1]. Cap the conical tube[2]. 2. Centrifuge the Zymo-Spin™ V-E Column/Reservoir at 1,000 x *g* for 5 minutes[3]. Discard the flow through[4]. 3. Add 9 ml **DNA Pre-Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir and centrifuge at 1,000 x *g* for 5 minutes[3]. Discard the flow through[4]. 4. Add 7 ml **g-DNA Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir and centrifuge at 1,000 x *g* for 5 minutes[3]. Discard the flow through.	1. Place the **Zymo-Spin™ V-E Column/Reservoir** onto a vacuum manifold[1]. 2. Transfer the lysate to the Zymo-Spin™ V-E Column/Reservoir and then turn on the vacuum source, set at ≥500 mm Hg. Allow all of the lysate to flow through the column. 3. With the vacuum off, add 9 ml **DNA Pre-Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir. Turn on the vacuum source and allow all of the buffer to flow through the column. 4. With the vacuum off, add 7 ml **g-DNA Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir. Turn on the vacuum source and allow all of the buffer to flow through the column.

5. Remove and discard the Reservoir, and place the Zymo-Spin™ V-E Column into a **Collection Tube**. Centrifuge at 12,000 x *g* for 1 minute in a microcentrifuge to remove residual wash buffer from the column[5].

6. Transfer the Zymo-Spin™ V-E Column in a new **Collection Tube** and add 200 µl **g-DNA Wash Buffer** to the column. Centrifuge at 12,000 x *g* for 1 minute in a microcentrifuge. Discard the flow through.

7. Transfer the Zymo-Spin™ V-E Column to a clean 1.5 ml microcentrifuge tube (not provided). Add 200 µl **DNA Elution Buffer**[6,7] to the column. Incubate for 5 minutes at room temperature, then centrifuge at 12,000 x *g* for 1 minute to elute the DNA[8]. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20 °C for future use.

Notes:

[1] Caution: Make sure the connection between the **Zymo-Spin™ Column** and the **Reservoir** is secure (finger tight) prior to centrifugation or vacuum.

[2] See the Troubleshooting guide for instructions on how to cap the tube.

[3] Centrifuge for 5 minutes or until all the lysate or wash buffer passes through the column.

[4] It is recommended to put the Zymo-Spin™ V-E Column/Reservoir in a clean 50 ml conical tube (not provided) after each centrifugation step.

[5] Leave the rotor cover off the microcentrifuge if clearance with the column top is a problem.

[6] For optimal elution efficiency, **DNA Elution Buffer** or water (pH is >6.0) can be heated to 60-70 °C. Also, extending incubation time and loading the eluate a second time can increase the total yield.

[7] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA.

[8] The total yield can be improved by eluting the DNA with **DNA Elution Buffer** or water pre-equilibrated to 60-70 °C. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can increase total yield as well.

Appendix A

Cell Monolayer Sample Preparation:

The following procedure is designed for up to 3 x 10^7 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 (**pg. 6**).

Guidelines for Monolayer Cell DNA Isolation:

Cell numbers (growth densities) can vary between different cell types. Table 2 (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 2: Culture Plate/Flask Growth Area (cm^2) and Cell Number

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

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 500 x *g* for 5 minutes. Discard the supernatant without disturbing the cell pellet. Continue from Step 1 of the Biological Fluids & Cells workflow (**pg. 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 (**pg. 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** for every 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 40 minutes for samples ≤ 1 ml and 2 hours for samples > 1 ml.
3. Continue from Step 3 of the Biological Fluids & Cells Workflow (**pg. 6**).

Solid Tissues

1. To each sample (≤125 mg) prepared according the **DNA/RNA Shield™** specifications, add ½ volume **Solid Tissue Buffer (Blue)** and 20 µl **Proteinase K**.

   Note: *Tissue samples should be mechanically homogenized for optimal extraction efficiency.*

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

   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 ≥ 12,000 x *g* for 1 minute in a microcentrifuge. Transfer aqueous supernatant to 15 ml conical tube.
4. Add 1 volume **Genomic Lysis Buffer** to the digested sample. Mix thoroughly or vortex for 10-15 seconds.
5. Continue from Step 1 of the DNA Purification Protocol (**pg. 7**).

Notes:

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

Notes:

[1] Additional **DNA Elution Buffer** may need to be purchased for this protocol (D3004-4-10, D3004-4-50).

Appendix C

Highly Nucleated Blood Samples:

1. Add up to 150 µl of nucleated blood to the following:

BioFluid & Cell Buffer (Red)	3 ml
Proteinase K	100 µl
DNA Elution Buffer[1] (or TE Solution)	3 ml

2. Mix thoroughly by vortexing for 15 seconds. Then incubate the tube at 55 °C for 1-3 hours or until solubilized.

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

3. Add 1 volume of **Genomic Lysis Buffer** to the tube and mix thoroughly by pipetting up and down followed 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. Continue from Step 1 of the DNA Purification Protocol (**pg 7**).

Appendix D

Hair and Feather Samples:

1. Place the sample (≤ 100 mg) in a 15 ml conical tube and add **Proteinase K** and freshly prepared DTT (dithiothreitol) (not provided) as follows:

Water	360 µl
Solid Tissue Buffer (Blue)	360 µl
DTT (1 M)	40 µl
Proteinase K	40 µl

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 1,600 µl **Genomic Lysis Buffer** to the tube and mix thoroughly by vortexing for 15 seconds. Centrifuge at 1,000 *x g* for 5 minutes to pellet insoluble debris[1].

4. Continue from Step 1 of the DNA Purification Protocol (**pg 7**).

Notes:

[1] Make sure the supernatant is free from insoluble debris, if not increase centrifugation time as needed.

Quick-DNA™ Midiprep Plus Kit
Quick Protocol
Catalog No. D4075

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Biological Fluids & Cells Protocol
Biological Fluids: ≤ 3 ml
Total DNA from whole blood, buffy coat, saliva, sputum, semen, etc. See the Instruction Manual page 2.
Cultured Cells: ≤ 3x10^7
Total DNA from *E. coli*, insect, or mammalian cells (e.g. HeLa cells, buccal cells, HEK-293 cells, etc).
Note: *Pellet cells and discard supernatant. Resuspend cell pellets using* ***DNA Elution Buffer*** *or an isotonic buffer (e.g. PBS):*
< 1 x 10^6 cells in 500 µl
1-3 x 10^7 cells in 1,000 µl
**Add 1,060 µl of Proteinase K Storage Buffer to each 20 mg tube of Proteinase K. Store at -20 °C after mixing.*

Biological Fluids & Cells

1. Add up to 3 ml sample to a 50 ml conical tube (not provided) and add:
   - 3 ml **BioFluid & Cell Buffer (Red)**
   - 100 µl **Proteinase K**

   Note: *For inputs < 3 ml biological fluid, proportionally decrease BioFluid & Cell Buffer (Red) and Proteinase K (See Table 1).*
2. Vortex for 15 seconds and then incubate the tube at 55 °C for:
   - 40 min if sample ≤ 1 ml
   - 2 hrs if sample ≤ 3 ml
3. Add 1 volume **Genomic Lysis Buffer** to the digested sample, ignoring volume contribution from the Proteinase K. Vortex for 15 seconds (See Table 1).

   Example: Add 6.0 ml Genomic Lysis Buffer to the 6.0 ml total volume digested sample.

Table 1: Quick Setup Guide

Sample Volume	500 µl	1 ml	2 ml	3 ml
BioFluid & Cell Buffer (Red)	500 µl	1 ml	2 ml	3 ml
Proteinase K	20 µl	30 µl	70 µl	100 µl
Mix thoroughly and incubate at 55 °C for: 40 min if sample ≤ 1 ml or 2 hrs if sample ≤ 3 ml				
Add Genomic Lysis Buffer	1.0 ml	2.0 ml	4.0 ml	6.0 ml

Solid Tissues Protocol
Solid Tissues: ≤ 125 mg
Total DNA from tail snips, ear punches, organ biopsies (brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.). For special sample types including, hair and feather, see the Instruction Manual page 3.
** Add 1,060 µl of Proteinase K Storage Buffer to each 20 mg tube of Proteinase K. Store at -20 °C after mixing.*

Solid Tissues

1. To a tissue sample (≤ 125 mg) in a microcentrifuge tube (not provided), add a solution of:
   - 480 µl Water
   - 480 µl **Solid Tissue Buffer (Blue)**
   - 40 µl **Proteinase K**
2. Vortex for 15 seconds and then incubate the tube at 55 °C for 1-3 hours or until tissue solubilizes. Mix thoroughly.

   Note: *To remove insoluble debris, centrifuge at 12,000 x g for 1 minute. Transfer aqueous supernatant to a 15 ml conical tube (not provided).*
3. Add 4 volumes **Genomic Lysis Buffer** to the supernatant. Vortex for 15 seconds.

   Example: *Add 4 ml Genomic Lysis Buffer to the 1 ml supernatant.*

DNA Purification Made Simple™

For the full Instruction Manual, visit
http://www.zymoresearch.com/m/D4075

Ver. 1.0.0

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Quick-DNA™ Midiprep Plus Kit
Quick Protocol

Catalog No. D4075

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DNA Purification Protocol

Centrifugation Protocol	*Vacuum Protocol*
1. Transfer the lysate to the **Zymo-Spin™ V-E Column/Reservoir** inserted in a 50 ml conical tube. Cap the conical tube. 2. Centrifuge the Zymo-Spin™ V-E Column/Reservoir at 1,000 x *g* for 5 minutes. Discard the flow through. 3. Add 9 ml **DNA Pre-Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir and centrifuge at 1,000 x *g* for 5 minutes. Discard the flow through. 4. Add 7 ml **g-DNA Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir and centrifuge at 1,000 x *g* for 5 minutes. Discard the flow through.	1. Place the **Zymo-Spin™ V-E Column/Reservoir** onto a vacuum manifold. 2. Transfer the lysate to the Zymo-Spin™ V-E Column/Reservoir and then turn on the vacuum source, set at ≥500 mm Hg. Allow all of the lysate to flow through the column. 3. With the vacuum off, add 9 ml **DNA Pre-Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir. Turn on the vacuum source and allow all of the buffer to flow through the column. 4. With the vacuum off, add 7 ml **g-DNA Wash Buffer** to the Zymo-Spin™ V-E Column/Reservoir. Turn on the vacuum source and allow all of the buffer to flow through the column.

5. Remove and discard the Reservoir, and place the Zymo-Spin™ V-E Column into a **Collection Tube**. Centrifuge at 12,000 x *g* for 1 minute in a microcentrifuge to remove residual wash buffer from the column.

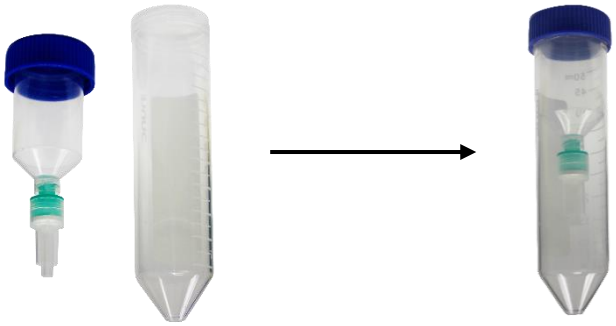
6. Transfer the Zymo-Spin™ V-E Column in a new **Collection Tube** and add 200 µl **g-DNA Wash Buffer** to the column. Centrifuge at 12,000 x *g* for 1 minute in a microcentrifuge. Discard the flow through.

7. Transfer the Zymo-Spin™ V-E Column to a clean 1.5 ml microcentrifuge tube (not provided). Add 200 µl **DNA Elution Buffer** to the column. Incubate for 5 minutes at room temperature, then centrifuge at 12,000 x *g* for 1 minute to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20 °C for future use.

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

Troubleshooting

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

Problem	*Possible Causes and Suggested Solutions*
How to Assemble the Zymo-Spin™ V-E Column with Reservoir into a 50 ml Conical Tube	 Snap the cap in the reservoir Screw cap into the 50 ml conical tube
How to Load Buffers into the Zymo-Spin™ V-E Column	 Insert the pipette tip at the top of the column and gently touch the matrix while adding the corresponding buffers
DNA Elution Guide	*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™ Midiprep 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).

Low DNA Yield	*Tissue Input* • For low DNA-containing tissues (e.g. muscle, etc.) using larger inputs will increase yields (up to 125 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. • Ensure the DNA Elution Buffer hydrates the matrix for 5 minutes at room temperature before centrifugation. *Elution Procedures* • 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.
Low DNA Purity	*Low $A_{260/230\ nm}$* • Excessive lysate can remain in the column after the loading step. To increase the purity ($A_{260/230\ nm}$) of the DNA the following steps can be taken. Before performing the first wash (**pg. 7**, Step 3), disconnect the Zymo-Spin™ V-E Column from the Reservoir and place the column in a 1.5 ml microcentrifuge tube (not provided). Centrifuge at 12,000 x *g* in a microcentrifuge for 1 minute. Discard the flow through, re-attach the Reservoir onto the Zymo-Spin™ V-E Column, and continue to Step 3 of the DNA Purification Protocol on **pg. 7**. • If the outside of the column gets splashed with lysate or wash buffer, wipe the outside of the column with a paper towel, pre-moistened with 70% ethanol. *Low $A_{260/280\ nm}$* • Check Procedural Errors and Incomplete Debris removal in the section "Low DNA Performance" below.
Low DNA Performance	*Procedural Errors* • Ensure the proper digestion buffer is used. See the Purification Guide on **pg. 5**. • Ensure the correct volume of Genomic Lysis Buffer is used. See the Quick Setup Guide on **pg. 6.** • Ensure the column tip does not touch the flow through, since the column tip can be contaminated with wash buffer flow through. Use a new 50 ml conical tube when instructed. • Insufficient centrifugation: Ensure the indicated centrifugation times and speeds are used. Increase the centrifugation time of the binding step or wash steps when needed to ensure complete buffer removal.

	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 times as needed until the sample completely solubilizes. Insoluble debris should be removed by centrifuging the sample at 12,000 x *g* for 1 minute in a microcentrifuge, and subsequent supernatant should be removed without disturbing the debris. *Tissue Input* • Make sure the lysate has passed completely through the matrix before proceeding to the wash steps. • Vortex samples longer after the addition of Genomic Lysis Buffer to ensure that the lysate is homogenous. • 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™ Midiprep 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 Lysis Buffer and corresponding digestion buffer is used. See the Sample Preparation Protocol and Quick Setup Guide on **pg. 6**. • 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
***Quick*-DNA™ Miniprep Plus Kit**	D4068 D4069	50 Preps 200 Preps
***Quick*-DNA™ Microprep Plus Kit**	D4074	50 Preps
***Quick*-DNA™ 96 Plus Kit**	D4070 D4071	2 x 96 Wells 4 x 96 Wells
***Quick*-DNA™ Midiprep Plus Kit**	D4075	25 Preps

For Individual Sale	Catalog No.	Amount
Proteinase K & Storage Buffer	D3001-2-5 D3001-2-20	5 mg set 20 mg set
BioFluid & Cell Buffer (Red)	D4068-1-12 D4068-1-45	12 ml 45 ml
Solid Tissue Buffer (Blue)	D4068-2-6 D4068-2-22	6 ml 22 ml
Genomic Lysis Buffer	D3004-1-50 D3004-1-100 D3004-1-150	50 ml 100 ml 150 ml
DNA Pre-Wash Buffer	D3004-5-50 D3004-5-250	50ml 250 ml
g-DNA Wash Buffer	D3004-2-100 D3004-2-200	100 ml 200 ml
DNA Elution Buffer	D3004-4-10 D3004-4-50	10 ml 50 ml
Zymo-Spin™ V-E Columns with Reservoir	C1029-25	25 columns/ reservoirs
Collection Tubes	C1001-50 C1001-500	50 tubes 500 tubes